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Petition for the Determination of Nonregulated Status for Improved Fatty Acid Profile MON 87705 Soybean

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

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Revised July 16, 2010

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Monsanto Petition Number: 09-SY-201U

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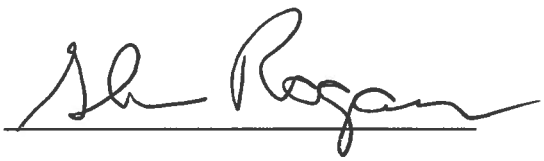
CERTIFICATION

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



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EXECUTIVE SUMMARY

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

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status in whole for the new biotechnology-derived soybean product, MON 87705, any progeny derived from crosses between MON 87705 and conventional soybean, and any progeny derived from crosses of MON 87705 with other biotechnology-derived soybean that has been granted nonregulated status under 7 CFR Part 340.

Product Description

Monsanto Company has developed biotechnology-derived soybean MON 87705 with an improved fatty acid (FA) profile that results in an oil that has enhanced nutritional characteristics, and improved suitability and stability for food and industrial uses. Currently commodity soybean oil requires hydrogenation to improve its stability for use in many foods given its high proportion of polyunsaturated fatty acids. Hydrogenation results in the formation of *trans* fatty acids that pose known coronary health risks. As food companies reformulated foods to replace *trans* fat-containing hydrogenated oils with healthier alternatives, they have faced challenges in finding high stability oils that are also relatively low in saturated fat.

Using the extensive information known regarding the fatty acid biosynthetic pathway in soybean, MON 87705 was developed to selectively down-regulate, in seed, two key enzymes involved in fatty acid biosynthesis. As a result, MON 87705 soybean oil is lower in saturated fats (6% vs. 15% of total fatty acids) and higher in monounsaturated 18:1 oleic acid (76% vs. 23% FA), with an associated decrease in the polyunsaturated 18:2 linoleic acid levels (10% vs. 53% FA) relative to commodity soybean. Consequently, MON 87705 soybean oil, with improved oxidative stability and lower saturated fats than currently available commodity soybean oil, is suitable for a range of food and industrial applications. In addition, soybean meal derived from MON 87705, which contains very low residual oil, is unchanged in composition relative to commodity soybean meal. MON 87705 also contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp*. strain CP4 (*cp4 epsps*) encoding the CP4 EPSPS protein that is expressed throughout the plant conferring tolerance to glyphosate, the active ingredient in the Roundup® family of agricultural herbicides.

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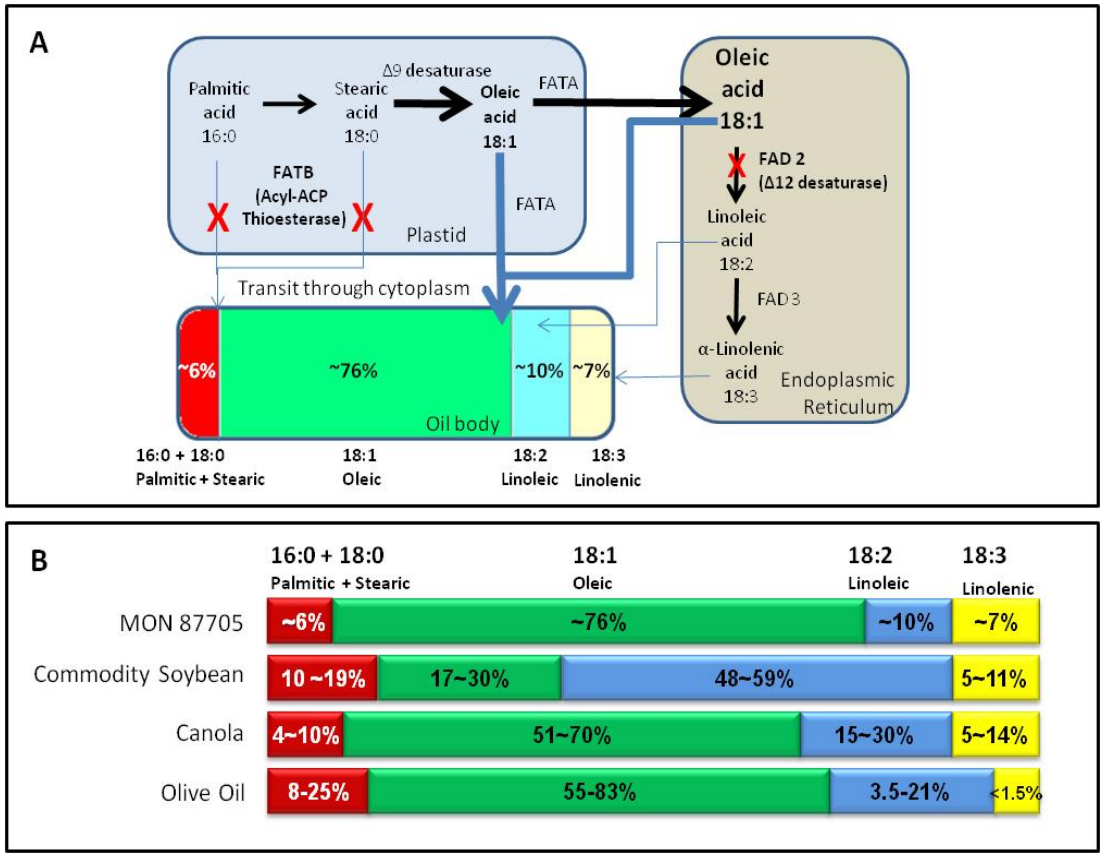
The improved fatty acid profile in MON 87705 soybean oil is achieved through the use of endogenous soybean (*Glycine max* L.) gene segments configured to suppress *FATB* and *FAD2* gene expression. MON 87705 contains *FATB1-A* and *FAD2-1A* gene segments under the control of a seed promoter, limiting oil composition modification to this tissue. The assembled gene transcript has an inverted repeat that produces double stranded RNA (dsRNA) that, via the RNA interference (RNAi) pathway, suppresses endogenous *FATB* and *FAD2* gene expression, thereby producing the desired fatty acid phenotype (see Figure below).



Schematic of the Soybean Fatty Acid Biosynthetic Pathway and Summary of Modified Fatty Acid Content in Soybean Oil Derived from MON 87705
Panel A: Schematic of the soybean fatty acid biosynthetic pathway
Panel B: MON 87705 soybean oil compared to commodity soybean oil and other vegetable oils

✕ indicates suppression of endogenous *FATB* and *FAD2* in MON 87705 seeds.

Acyl-acyl carrier protein (ACP) thioesterases (referred to herein as FATB enzymes) are localized in plastids and hydrolyze saturated fatty acids from the ACP-fatty acid moiety. The suppression of *FATB* results in a decrease in the transport of the saturated fats out of the plastid, thus retaining their availability for desaturation to 18:1 oleic acid (see Figure above). Therefore, suppression of *FATB* decreases saturated fat content in the oil as well as increasing oleic acid. Subsequently, this increased amount of oleic acid is either delivered to the oil body or endoplasmic reticulum for further desaturation. Delta-12

desaturases (referred to as FAD2 enzymes) desaturate 18:1 oleic acid to 18:2 linoleic acid. The suppression of *FAD2* in soybean seed causes reduced desaturation of oleic to linoleic acid thus contributing further to the increase in oleic while reducing linoleic acid content in the oil. Therefore, the overall result of the suppression of these two enzymes is a reduction in saturated 16:0 palmitic and 18:0 stearic fatty acids, an increase in monounsaturated 18:1 oleic acid, and lower levels of polyunsaturated 18:2 linoleic acid relative to commodity soybean.

The MON 87705 soybean oil improved fatty acid profile provides new options for food companies interested in the formulation of lower saturated fat foods. Soybean oil is comprised primarily of five major fatty acids: saturated fatty acids 16:0 palmitic and 18:0 stearic acids, monounsaturated 18:1 oleic acid, and the polyunsaturated fatty acids, 18:2 linoleic, and 18:3 linolenic acids. These five major fatty acids have very different oxidative stabilities and chemical functionalities. Conventional soybean oil typically contains 60-65% polyunsaturated fatty acids, mostly in the form of linoleic acid. This composition makes soybean oil unsuitable for certain food applications since the high concentrations of polyunsaturated fatty acids in the oil are susceptible to oxidation and degradation at high temperature. Therefore, hydrogenation of soybean oil is necessary to reduce levels of polyunsaturated fatty acids by converting them to more saturated fatty acids resulting in higher stability oil suitable for a range of food uses. The hydrogenation process used to reduce polyunsaturated fatty acids and increase the stability of soybean oil produces *trans* fatty acids that are linked to increased cardiovascular risk due to the elevation of low-density lipoproteins (LDL) and reduced levels of high-density lipoproteins (HDL). Because MON 87705 soybean oil has a reduced level of polyunsaturated fatty acids, it has higher oxidative stability without the need for hydrogenation, while also containing a lower level of saturated fats. Saturated fats, notably palmitic acid, have also been shown to contribute to cardiovascular disease and other chronic diseases. As a result, the reduced saturated fat levels in MON 87705 soybean oil can also positively impact the goal of keeping human dietary consumption of saturated fats below 10% of total energy intake[1].

In addition to providing improved formulation options for food companies, the reduction in saturated fats and increased oxidative stability of MON 87705 soybean oil increases suitability for biodiesel and other industrial applications. Low saturated fats and high (>70%) oleic acid levels are key attributes for vegetable oils targeted for biodiesel and industrial uses because of improved cold weather performance, improved stability, and reduced nitrous oxide emissions.

Therefore, the fatty acid profile of MON 87705 soybean oil, with an increase in monounsaturated oleic acid and decreases in saturated fats and polyunsaturated fatty acids (17% vs. 60% FA), significantly improves the soybean oil functionality and nutritional value. MON 87705 will be bred with current commercial low linolenic acid soybean varieties that will further enhance the oxidative stability of soybean oil.

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[1] Department of Health and Human Services, *Dietary Guidelines for Americans*, 2005. www.health.gov/dietaryguidelines [Accessed June 7, 2009]

Studies Confirm the Lack of Plant Pest Potential of MON 87705

The data and information presented in this petition demonstrate MON 87705 is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of the intended modifications to oil composition and tolerance to glyphosate. Moreover, the data presented show MON 87705 is unlikely to pose an increased plant pest risk, including weediness or adverse environmental impact, compared to conventional soybean. The food, feed and environmental safety of MON 87705 was confirmed based on multiple, well established lines of evidence:

1. Soybean is a familiar crop that does not possess any of the attributes commonly associated with weeds, has a history of safe consumption, and serves as an appropriate basis of comparison.
2. A detailed molecular characterization of the inserted DNA demonstrated a single, intact copy of the transgenic insert in a single locus within the soybean genome. This insert contains the *FATB* and *FAD2* suppression cassette and the *cp4 epsps* expression cassette.
3. The inverted repeat encoded by the *FATB* and *FAD2* suppression cassette in MON 87705 does not code for any protein. The RNA-based suppression of *FATB* and *FAD2* soybean genes in MON 87705 is mediated by double stranded RNA (dsRNA) molecules. Double stranded RNAs are commonly used by eukaryotes, including plants, for endogenous gene suppression and pose no novel risks from a food, feed or environmental perspective. Nucleic acids, such as RNA, have a long history of safe consumption and are considered GRAS by the U.S. Food and Drug Administration.
4. The only introduced protein produced in MON 87705 is CP4 EPSPS. Data confirmed the CP4 EPSPS protein in MON 87705 is unlikely to be a toxin or allergen based on extensive information collected and evaluations performed. The CP4 EPSPS protein in MON 87705 has the same functional and enzymatic activity as the CP4 EPSPS in other Roundup Ready® crops previously deregulated by USDA, and is structurally homologous to EPSPSs naturally present in other crops.
5. A compositional assessment confirmed that, except for intended fatty acid changes, MON 87705 seed and forage are compositionally equivalent to seed and forage of conventional soybean. MON 87705 soybean oil does not contain any new fatty acids that are not already present in commodity soybean oil, and the fatty acid profile of MON 87705 soybean oil is similar to many other commercial oils currently available.
6. An extensive evaluation of MON 87705 phenotypic and agronomic characteristics and environmental interactions demonstrate MON 87705 shows no increased plant pest risk potential compared to conventional soybean.

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7. An assessment of potential impact to nontarget organisms (NTOs) and endangered species indicates MON 87705 is unlikely to have adverse effects on these organisms compared to conventional soybean under normal agricultural practices.
8. An evaluation of MON 87705 on current cultivation and management practices for soybean concluded deregulation of MON 87705 will not significantly impact current soybean agronomic practices and land use.

Soybean is a Familiar Crop Lacking Weedy Characteristics

There is a long standing history of safe consumption of conventional soybean and its oil, as soybean is the most prevalently grown oilseed in the world, with approximately 222.1 million metric tons of harvested seed (MMT) produced in 2007, representing 56% of world oilseed seed production that year. Soybean is grown as a commercial crop in over 35 countries and domestication of this crop can be traced back to approximately 1000 B.C. A major food use for soybean is purified oil, for use in margarines, shortenings, cooking, and salad oils.

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 major weed references, nor is it present on the lists of noxious weed species distributed by the federal government. Soybean does not possess any of the attributes commonly associated with weeds, such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. Due to a pronounced 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. In addition, since wild populations of *Glycine* species are not known to exist in the U.S., the potential does not exist for MON 87705 to outcross to wild or weedy relatives and alter their weediness potential.

The genetic background of MON 87705 was matched with that of an appropriate control, so the effect of the genetic insertion and the presence of the CP4 EPSPS protein could be assessed in an unbiased manner. Since MON 87705 was derived from the A3525 conventional variety, it was deemed appropriate to use the nontransformed A3525 as the control variety because its use would minimize the potential bias in subsequent comparative assessments.

Molecular Characterization Verified the Integrity and Stability of the Inserted DNA

MON 87705 was produced by *Agrobacterium*-mediated transformation of soybean with the binary vector PV-GMPQ/HT4404 that contains two T-DNAs. T-DNA I and T-DNA II both contain DNA segments designed to suppress endogenous *FAD2* and *FATB* genes which encode for two key enzymes in the soybean fatty acid biosynthetic pathway. T-DNA I also contains a *cp4 epsps* expression cassette. The partial suppression cassette in T-DNA I contains the sense segments of the *FAD2-1A* intron and *FATB1-A* 5'

untranslated region (UTR) which are under the regulation of the *7Sα'* seed promoter. T-DNA II contains a partial suppression cassette that contains the antisense segment of *FAD2-1A* and *FATB1-A*.

During plant transformation, the two T-DNAs (T-DNA I and T-DNA II, respectively) co-integrated into one locus in the soybean genome. The cointegration of the T-DNAs in this configuration creates an insert containing a single *cp4 epsps* expression cassette and a single *FAD2-1A* and *FATB1-A* suppression cassette. The *in planta* assembled suppression cassette includes an inverted repeat that results in suppression of endogenous *FAD2* and *FATB* RNA expression.

Molecular characterization of MON 87705 by Southern blot demonstrates there is one copy of each T-DNA insert within the same locus of integration. Backbone sequences from plasmid PV-GMPQ/HT4404 were not detected in the genome of MON 87705. Additionally, the data confirm the organization and sequence of the insert, demonstrate the stability of the insert over several generations, and that the genomic DNA sequences flanking the 5' and 3' ends of the insert are native to the soybean genome.

RNA-Based Suppression Technology in MON 87705 Does not Pose Unique Safety Risks

The RNA-based suppression of *FATB* and *FAD2* soybean genes in MON 87705 is mediated by dsRNA molecules. Double stranded RNAs are commonly found in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids. Nucleic acids have a long history of safe consumption and are considered GRAS by the U.S. Food and Drug Administration. There is no evidence to suggest dietary consumption of RNA is associated with toxicity or allergenicity. Moreover, analysis of the DNA segments encoding this dsRNA showed they lack the sequences required for translation initiation and protein synthesis. The production of a protein from the dsRNA encoded by the insert in MON 87705 is highly unlikely. Several biotechnology-derived plant products previously deregulated by APHIS were developed using RNA-based suppression mechanisms, including virus-resistant papaya and squash, high oleic soybean, FLAVR SAVR tomatoes, and plum trees resistant to Plum pox virus. Based on this information, it is concluded that the inserted DNA and resulting dsRNA are safe and unlikely to produce a protein or polypeptide. As a result, the RNA-based suppression technology used in MON 87705 poses no novel risks from a food, feed or environmental perspective.

Data Confirm CP4 EPSPS Protein Safety

A multistep approach was used to characterize and assess the safety of the CP4 EPSPS protein expressed in MON 87705. This detailed characterization confirms the CP4 EPSPS protein is safe for human and animal consumption. The assessment involved: 1) characterizing the physicochemical and functional properties of the protein; 2) quantifying protein levels in MON 87705 plant tissues; 3) examining the similarity of the CP4 EPSPS protein to known allergens, toxins and other biologically-active proteins known to have adverse effects on mammals; 4) evaluating the digestibility of CP4 EPSPS protein in simulated gastrointestinal fluids; 5) documenting the history of safe consumption CP4 EPSPS protein; and 6) investigating potential mammalian toxicity through an oral gavage assay. Results confirm that CP4 EPSPS is expressed in all tissues

collected, including root, forage, seed, and leaf tissues at four developmental stages. CP4 EPSPS has no relevant amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which can have adverse effects on mammals. Studies utilizing the CP4 EPSPS protein revealed that it degrades rapidly in simulated gastric and intestinal fluids and mouse acute oral toxicity evaluations demonstrate the protein is not acutely toxic and does not cause any adverse effect, even at the highest dose tested. The safety assessment supports the conclusion that dietary exposure to CP4 EPSPS protein derived from MON 87705 poses no meaningful risk to human or animal health.

MON 87705 is Compositionally Equivalent to Conventional Soybean, Except for the Intended Fatty Acid Changes

Detailed compositional and nutritional comparisons were conducted to assess whether levels of nutrients, anti-nutrients, and key secondary metabolites in seed and forage derived from MON 87705 are comparable to those in a conventional soybean control and several commercially available reference varieties. The analysis included proximates (protein, fat, carbohydrates, fiber, ash, moisture) in seed and forage, and fatty acids, amino acids, vitamin E, and antinutrients in seed. Composition data were analyzed statistically for all components and their biological and nutritional significance were evaluated. Statistical analyses were conducted on data from a combination of all sites (combined-site) and data from each of five individual sites (individual-site). If a significant difference ($p<0.05$) in an analyte was detected between MON 87705 and its conventional control in the combined-site comparison, an analysis including reproducibility across individual sites, magnitude of differences, and comparisons of MON 87705 mean analyte values to the 99% tolerance interval for the population of commercial conventional soybean varieties grown concurrently at the same time and field sites and published values was made to assess whether the difference was biologically meaningful from a food and feed safety or nutritional perspective. The compositional analysis confirmed that MON 87705 seed had the intended fatty acid composition, while the other components analyzed in MON 87705 seed and forage were considered to be compositionally equivalent to conventional soybean. Moreover, no new fatty acids beyond those presently found in soybean were detected in MON 87705.

Of the nine fatty acids that were analyzed statistically, significant differences ($p<0.05$) were observed for seven fatty acids in the combined-site analysis. As intended, MON 87705 seed had significantly ($p<0.05$) lower 16:0 palmitic and 18:0 stearic acid levels (combined saturates 5.7%), higher 18:1 oleic acid (76.5%) and an associated decrease in 18:2 linoleic acid (10.1%) compared to conventional soybean. Differences in these four fatty acids were consistently observed at each of the individual sites and levels fell within the intended fatty acid ranges. A combined-site statistical difference ($p<0.05$) between MON 87705 and the conventional control was observed in the levels of 18:3 linolenic acid. The decrease in 18:3 linolenic acid is expected given that it is produced from 18:2 linoleic acid which was reduced by the suppression of the *FAD2* gene. Examination of the reproducibility within sites shows the levels of 18:3 linolenic acid were significantly lower than the soybean control in four of five individual-site analyses, with the absolute magnitude of the differences being small (<1.5% total FA content). In addition, all the mean levels of 18:3 linolenic acid in MON 87705 seed from the combined-site and individual-site analyses were well within the 99% tolerance interval,

and therefore these differences are not considered biologically relevant compositional changes. Combined-site statistical differences between MON 87705 and the conventional control were also observed in the levels of two minor fatty acids, 20:0 arachidic acid, and 20:1 eicosenoic acid. Examination of the reproducibility within sites shows the absolute magnitude of the differences for these two minor fatty acids was very small (<0.19% of total FA) and mean values and ranges in MON 87705 seed were within either the 99% tolerance interval for the population of the conventional reference varieties or the values reported in International Life Science Institute-Crop Composition Database (ILSI-CCD) and published literature. No difference was observed between MON 87705 and the conventional control for the remaining two minor fatty acids (22:0 behenic acid and 24:0 lignoceric acid) evaluated. Thus, apart from the intended or expected differences in fatty acid levels, there were no other biologically meaningful differences in the levels of other fatty acids.

Combined-site analyses of both forage and seed samples for non-fatty acid analytes showed no statistically significant difference ($p>0.05$) between MON 87705 and the conventional control for 37 of 41 analyte comparisons. Statistical comparisons between MON 87705 and the conventional control for the presence of other components showed that three analytes in soybean seed (cystine, arginine and total fat) and one analyte in forage (ash) were significantly different ($p<0.05$) in the combined-site analysis. For these four analytes where differences were noted ($p<0.05$), the absolute magnitude of differences between MON 87705 and the conventional soybean control were generally low (<1.1% dw), were not observed consistently across individual sites (individual-site analyses), and mean values for MON 87705 were within the calculated 99% tolerance interval for the population of conventional reference varieties. Harvested seed and forage analytical component values also were comparable to values reported in the ILSI-CCD and/or published literature, further supporting the conclusion that harvested seed and forage from MON 87705 are compositionally equivalent to those of conventional soybean. Therefore, it is concluded that the statistical differences observed are not biologically meaningful.

In addition to the compositional analysis of seed and forage, four soybean processed fractions (refined oil, meal, lecithin, and protein isolate) were produced from MON 87705 and conventional control seed and subjected to compositional analysis in accordance with OECD guidelines. As expected, and consistent with results obtained for seed fatty acid levels, the intended fatty acid changes were observed in the refined oil fraction. As in seed, levels of several other less abundant fatty acids were also significantly different ($p<0.05$) between the refined soybean oil fractions from MON 87705 and the conventional soybean control. For these analytes, MON 87705 mean values fell within the 99% tolerance intervals for the reference varieties and/or were comparable to published literature ranges for conventional soybean oil. Differences in the levels of two other minor fatty acids, likely formed by spontaneous isomerization during the oil refining process, were also observed. Since these differences are not considered biologically relevant, as they likely are an artifact of the refining process. Apart from the intended fatty acid changes in the oil, the composition of the soybean processed fractions from MON 87705 is equivalent to the composition of the soybean processed fractions from the conventional soybean control. Thus, the processed fractions

from MON 87705 are as safe and nutritious as the processed fractions from conventional soybean.

The compositional analysis confirmed that MON 87705 seed had the intended fatty acid profile. In all compositional and nutritional comparisons of MON 87705 to its conventional control where a significant difference ($p<0.05$) was detected, other than for intended fatty acid changes, an analysis including reproducibility across individual sites, magnitude of differences, and comparisons of mean test analyte values to the 99% tolerance interval and published values, indicated that differences observed were not biologically meaningful from a food and feed safety or nutritional perspective. Therefore, except for the intended fatty acid changes, the compositional and nutritional assessment of MON 87705 supports the conclusion that seed and forage and key processed fractions produced from MON 87705 are compositionally equivalent to those of conventional soybean in accordance with OECD guidelines.

MON 87705 Does Not Change Soybean Plant Pest Potential or Environmental Interactions

The phenotypic, agronomic, and environmental interaction assessment indicates that MON 87705 is comparable to the parental conventional soybean control, A3525, which has background genetics similar to MON 87705, but lacks the introduced traits. Thus, MON 87705 is unlikely to have changed plant pest risk potential or environmental impact compared to conventional soybean. An important element in assessing plant pest risk potential and environmental impact of MON 87705 is to compare MON 87705 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 known to experts. 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 appropriate conventional counterpart. The MON 87705 characteristics assessed include: seed dormancy and germination, pollen morphology, and symbiont interactions conducted in the laboratory and greenhouse, and plant phenotypic observations and environmental interaction evaluations conducted in the field.

Seed dormancy and germination characterization indicated that MON 87705 seed had germination characteristics similar to that of the conventional soybean control. In particular, the lack 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 87705 compared to conventional soybean for germination and dormancy characteristics. For pollen characteristics and symbiont interactions, there were no statistically significant differences ($p<0.05$) observed for any of the parameters measured, including pollen viability, nodule dry weight, and shoot total nitrogen. Collectively, these results support the conclusion that MON 87705 is not likely to exhibit increased weed potential compared to conventional soybean.

The field evaluation of phenotypic, agronomic, and ecological characteristics of MON 87705 also supports the conclusion that MON 87705 is not likely to pose an

increased weed or plant pest potential compared to conventional soybean. The evaluations were conducted at 17 replicated field sites across U.S. soybean production regions. These assessments included 14 plant growth and development characteristics, as well as observations for plant-insect and plant-disease interactions and plant responses to abiotic stressors. The observed phenotypic characteristics were comparable between MON 87705 and the conventional soybean control. Across sites, data show no statistically significant differences between MON 87705 and the control for seedling vigor, plant height, lodging, pod shattering, seed moisture, test weight, or yield. Flower color and plant pubescence data were categorical and were not statistically analyzed; however, at each site, all plants of MON 87705 and the control had purple flowers and pubescence as expected.

Four statistically significant differences ($p<0.05$) were detected between MON 87705 and the control in the combined-site analysis. MON 87705 was lower than the control for early stand count, final stand count, the weight of 100 seeds and flowered approximately one day later than the control. MON 87705 and the control were within the same range of plant growth stages for 113 out of the 114 growth stage observations among the sites, and the single different observation at one site, was within the range of growth stages observed for the reference varieties. None of these differences were considered biologically meaningful in terms of increased weed potential.

In an individual site assessment of plant response to abiotic stress, disease damage, and arthropod damage, no differences were observed between MON 87705 and the control for 574 of 579 comparisons (including 167 abiotic stress response, 206 disease damage, and 206 arthropod damage comparisons) among all observations at the 17 sites. The five observed differences were in the disease and arthropod damage categories. For each of the five observed differences, the severity of damage in MON 87705 was within the range of the reference soybean varieties, and were not consistently observed across sites. Therefore, they were not considered biologically meaningful in terms of increased weed potential.

In an assessment of pest and beneficial arthropod abundance, no statistically significant differences ($p> 0.05$) were detected between MON 87705 and the control for 95 out of 96 comparisons (including 46 arthropod pest comparisons and 50 beneficial arthropod comparisons) among the collections at the four sites. The single statistically significant difference was for bean leaf beetle in a single collection time from a single site, where the mean abundance value from MON 87705 plots was lower than the conventional control, yet fell within the reference range. The differences in pest and beneficial arthropod abundance were not indicative of a consistent plant response associated with the traits and are unlikely to be biologically meaningful in terms of plant pest potential or environmental impact of MON 87705 compared to conventional soybean.

The plant phenotypic and ecological interaction parameters evaluated were used to characterize the plant and its interactions with the environment, and to assess the plant pest or weed potential of MON 87705 compared to the conventional soybean control. An analysis based on the weight of the evidence, including reproducibility, magnitude and direction of a difference, and comparison to reference ranges of the detected differences ($p<0.05$) found in the evaluation of phenotypic, agronomic and environmental characteristics of MON 87705 compared to the conventional soybean control supports the

conclusion that MON 87705 is not likely to increase weed or plant pest potential or to have a biologically meaningful change in terms of environmental impact potential.

MON 87705 Will Not Adversely Affect NTOs or Threatened or Endangered Species

Evaluation of the impacts of MON 87705 on NTOs is a component of the plant pest risk assessment. Assessment of the expected differences between MON 87705 and conventional soybean included the presence of the inserted genes, the expression of the CP4 EPSPS protein, and the improved fatty acid profile in MON 87705 seed. The nature of MON 87705 as a product with no pesticidal activity leads to a conclusion that all exposed organisms are considered to be NTOs. The environmental assessment of MON 87705 indicates that MON 87705 poses no adverse effect on NTOs or endangered species under normal agricultural practices. The environmental interactions evaluation included data collected in the phenotypic studies on plant-insect, plant-disease, and plant-environment interactions. The results of this assessment indicated the presence of the CP4 EPSPS protein and improved fatty acid profile in seed did not unexpectedly alter plant-insect interactions, including beneficial arthropods and insect pests, or alter disease susceptibility of MON 87705 compared to conventional soybean.

The fatty acids present in MON 87705 seed are widely prevalent in the environment. Fatty acids play a key role in metabolic energy storage and as components of phospholipids, which are essential for cellular membrane formation and function. As natural components of the plant and animal world, these fatty acids are not expected to accumulate, persist or be detrimental to the environment. In addition, the improved fatty acid profile in MON 87705 seed results in an oil that is very similar to seed fatty acid profiles in widely cultivated crops, such as canola (*Brassica napus* L. and *B. campestris* L.), thus establishing a history of safe environmental exposure.

The naturally glyphosate-tolerant EPSPS protein from an *Agrobacterium* sp., CP4 EPSPS, has been introduced into several conventional crops, such as soybean, corn and cotton, to provide tolerance to glyphosate, the active ingredient in the Roundup family of herbicides. There is no toxicity associated with this family of proteins, and since they are ubiquitous in plants and microorganisms, they have a history of safety in the environment. The CP4 EPSPS protein is nontoxic to animals including mammals, birds, and arthropods. Lack of hazard for MON 87705 was established through a combination of biochemical information and experimental data demonstrating the existence of no reasonable mechanism for harm.

Therefore, the assessment considered pertinent product characterization information, information from the protein safety assessments, the history of environmental exposure to these fatty acids, and results from the environmental interaction assessment. Taken together, these data support the conclusion that MON 87705 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 87705 to outcross with sexually compatible species, including threatened or endangered 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 to 6.3%) in adjacent plants. Furthermore, in the rare event when cross-pollination does occur, MON 87705 and its progeny would not have a significant environmental impact, because evaluations have shown the improved fatty acid profile and glyphosate-tolerance traits in MON 87705 have not enhanced weediness or plant-pest potential relative to conventional soybean. Therefore, the environmental consequence of pollen transfer from MON 87705 to other *Glycine* species is considered negligible.

Deregulation of MON 87705 Will Not Significantly Impact Soybean Agronomic Practices or Land Use

Soybean fields are typically highly managed agricultural areas that can be expected to be dedicated to crop production for many years. MON 87705 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 87705 on current cultivation and management practices for soybean. Except for the intended modification in fatty acid profile and glyphosate tolerance, MON 87705 is no different from conventional soybean in its agronomic, phenotypic, ecological, characteristics and has the same levels of resistance to insects and diseases as current commercial soybean. The introduction of MON 87705 provides growers the means to produce a highly valued oil for use in multiple food and industrial applications. Based on these considerations, there is no apparent potential for significant impact on agronomic practices or land use.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 87705 is not likely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87705 and any progeny derived from crosses between MON 87705 and conventional soybean or deregulated biotechnology-derived soybean be granted nonregulated status under 7 CFR Part 340.

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ABBREVIATIONS 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 carboxylterminal β-conglycinin coding region with the termination codon and the polyadenylation sequence
35S	Enhancer sequences from the promoter of the Figwort Mosaic virus (FMV) 35S RNA
aadA	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7
AA	Amino acid
ACP	Acyl carrier protein
AD_2009	Allergen gliadin and gluten proteins sequence database
ADF	Acid detergent fiber
AI	Adequate Intake
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
AOCS	American Oil Chemists Society
AOSA	Association of Official Seed Analysts
AOSCA	Association of Official Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service
APS	Analytical protein standard
ASA	America Soybean Association
bp	Base pair
B. japonicum	*Bradyrhizobium japonicum*
B-Left Border	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA
B-Right Border	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA
BSA	Bovine serum albumin
ºC	Degree Celsius
CAST	Council for Agricultural Science and Technology, USDA
CBI	Confidential business information

* 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*.

CdT	Calera de Tango Maipo Province, Chile
CES	Cooperative Extension Services
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
CI	Confidence interval
COA	Certificate of analysis
COC	Chain of custody
CP(A)	Buffer Containing 50 mM MES, pH 5.8, 10% glycerol (v:v), 1 mM benzamidine-HCl and 1 mM DTT
CP4	*Agrobacterium* sp strain CP4
cp4 epsps	coding sequence for the CP4 EPSPS Protein
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp., strain CP4
CPB	Cartagena Protocol on Biosafety
cpm	Counts per minute
CS-*cp4 epsps*	Codon modified coding sequence of the *aro*A gene from *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein
CS-*rop*	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*.
CSFII	Continuing Survey of Food Intakes by Individuals
CTAB	Hexadecyltrimethylammonium bromide
CTP	Chloroplast transit peptide
α-Cyano	α-cyano-4-hydroxycinnamic acid
Da	Dalton
DAP	Days after planting
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
DEEM-FCID	Dietary exposure evaluation model-food commodity intake database
DHB	2,5-Dihydroxybenzoic Acid
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
dsRNA	Double-stranded RNA
DTT	Dithiothreitol
DW	Dry weight
DWCF	Dry weight conversion factor
dwt	Dry weight of tissue
ECL	Enhanced chemiluminescence
E. coli	*Escherichia coli*

EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
E-Score	Expectation score
EU	European Union
FA	Fatty acid
FAARP	Food Allergy Research and Resource Program Database
FAD2	*Glycine max* gene for Δ-12 desaturase
FAD2-1A	The *Glycine max FAD2-1A* gene encoding the delta-12 desaturase
FAD2-1A^{P}	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene encoding the delta-12 desaturase which forms part of the suppression cassette
FAME	fatty acid methyl ester
FASTA	Algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences
FATB	*Glycine max* gene for Palmitoyl-ACP Thioesterase
FATB1-A	The *Glycine max FATB1-A* gene encoding the palmitoyl acyl carrier protein thioesterase
FATB1-A^{P}	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase which forms part of the suppression cassette
FDA	U.S. Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FMV	Figwort mosaic virus
FONSI	Finding of No Significant Impact
FW	Fresh weight
fwt	Fresh weight of tissue
GenBank	A public genetic database maintained by the National Center for Biotechnology Information at the National Institutes of Health, Bethesda, MD, USA
GI	Gene sequence identification number
GLP	Good Laboratory Practice
GLP-T	GLP Technologies
GRAS	Generarlly Recognized As Safe
GRR	Monsanto Company Guidelines for Keeping Research Records
HCl	Hydrochloric Acid

HDL	High-density lipoprotein
HRP	Horseradish peroxidase
H.U.	hemagglutinating unit
IDP	Identity preserved
IgG	Immunoglobulin G
ILSI	International Life Sciences Institute
ILSI-CCD	International Life Sciences Institute-Crop Composition Database
ISO	International Organization for Standardization
I-*Tsf1*	Intron from the *Tsf1* gene of *Aradidopsis thaliana* encoding elongation factor EF-1 alpha
kb	Kilo bases
kDa	Kilodaltons
KCl	Potassium Chloride
LB	Laemmli buffer
LDL	Low-density lipoprotein
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
L-*Tsf1*	5′ untranslated leader (exon1) from the *Tsf1* gene of *Aradidopsis thaliana* encoding elongation factor EF-1 alpha
MAFF	Ministry of Agriculture, Forestry and Fisheries, Japan
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MEEC	Maximum expected environmental concentration
MEL	Melipilla, Melipilla Province, Chile
MES	2-[N-Morpholino] Ethanesulfonic Acid
MH^+	Protonated mass ion
MHLW	Ministry of Health, Labor and Welfare, Japan
mM	Millimolar
MMT	Million metric tones
MOE	Margin of exposure
MRMP	Monsanto Resistance Management Plan
MS	Mass Spectrometry
MSL	Monsanto Scientific Literature
MTSA	Monsanto Technology Stewardship Agreement
MUFA	Monounsaturated fatty acid
MW	Molecular weight

MWCO	Molecular weight cutoff
MWM	Molecular weight marker
N/A	Not applicable
NCBI	National Center of Biotechnology Information at the National Institutes of Health, Bethesda, MD, USA
NDF	Neutral detergent fiber
NEPA	National Environmental Policy Act
NFDM	Non-fat dried milk
NHANES	National Health and Nutrition Examination Survey
NIST	National Institute of Standards and Technology
NMWC	Nominal molecular weight cut-off
NOAEL	No observed adverse effect level
NOP	National organic program
nt	Nucleotide
NTO(s)	Nontarget organism(s)
OECD	Organization for Economic Co-operation and Development
OR	Origin of replication
OR-*ori-PBR322*	Origin of replication from pBR322 for maintenance of plasmid in *E. coli*
OR-*ori-V*	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2
OSL	Overseason leaf
P-*7Sα′*	Promoter and leader from the *Sphas1* gene of *Glycine max* encoding beta-conglycinin storage protein (alpha′-bcsp) that directs transcription in seed
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
PEP	Phosphoenolpyruvate
P-*FMV/Tsf1*	Chimeric promoter consisting of enhancer sequences from the promoter of Figwort Mosaic Virus (FMV) *35S* RNA combined with promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha
P*i*	Inorganic Phosphate
PIP	Plant-incorporated protectant
PMSF	Phenylmethanesulfonyl fluoride
polyA$^+$ RNA	polyA enriched RNA
PPA	Plant Protection Act

PRESS	predicted residual sums of squares
PS(A)	Buffer Containing 50 mM Tris-HCl, pH 7.5, 1 mM DTT, 10% glycerol (v:v), 1.5 M $(NH_4)2SO_4$
PRT_2009	A protein sequence database derived from GenBank release 169
PTH	Phenylthiohydantoin
PUFA	Polyunsaturated fatty acid
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpolypyrrolidone
PV-GMPQ/HT4404	Plasmid used to transform the soybean genome to produce MON 87705
QS(A)	Buffer Containing 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM benzamidine-HCl, 4 mM DTT)
QAU	quality assurance unit
QUI	Quilapilun, Cachapoal Province, Chile
RAN	Rancagua, Cachapoal Province, Chile
RBD	refined, bleached, and deodorized
RCB	Randomized complete block
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*
RNAi	RNA interference
rop	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*
S3P	Shikimate-3-phosphate
SAP	Shrimp Alkaline Phosphatase
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
SFR	San Fernando, Colchagua Province, Chile
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SOP	Standard operating procedure
T/C/R	Test/Control/Reference
TD	toasted and defatted
TDF	Total dietary fiber

T-DNA I	Transfer DNA I
T-DNA II	Transfer DNA II
T-DNA	Transferred DNA
T-*E9*	3′ untranslated region of the pea *rbcS2* gene which functions to direct polyadenylation of the mRNA
TFA	*Trans* fatty acid(s)
T-*H6*	3′ untranslated sequence of the *H6* gene from *Gossypium barbadense* encoding a protein involved in secondary cell wall
TES	Threatened or endangered species
TIU	Trypsin inhibitor units
TMB	3,3’,5,5’-tetramethylbenzidene
TOX_2009	Toxin protein sequence database
Tris	Tris (hydroxymethyl) aminomethane
tRNA	Transfer RNA
TS-*CTP2*	Targeting sequence from the gene *shk*G encoding the transit peptide region of *Arabidopsis thaliana* EPSPS that functions to direct transport of the CP4 EPSPS protein to the chloroplasts
TSSP	Tissue-specific site pool
TUG	Technology Use Guide
Tween-20	Polyoxyethylenesorbitan monolaurate
Tz	Tetrazolium
U	Unit (of enzyme activity)
U.S.	United States of America
USDA	United States Department of Agriculture
USDA-APHIS	U.S. Department of Agriculture – Animal and Plant Health Inspection Service
USDA-ARS	United States Department of Agriculture – Agricultural Research Service
USDA-ERS	U.S. Department of Agriculture – Economic Research Service
USDA-GRIN	United States Department of Agriculture – Germplasm Resources Information Network
USDA-NASS	U.S. Department of Agriculture – National Agricultural Statistics Service
USDA-NSHS	U.S. Department of Agriculture – National Seed Health System
USFWS	U.S. Fish and Wildlife Service
UTR	Untranslated region
V	Volts
VOI	Verification of Identity
WPEP	Weed Performance Evaluation Program
v/v	Volume per volume

wt	weight
w/v	Weight per volume

I. RATIONALE FOR THE DEVELOPMENT OF MON 87705

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

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

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status in whole for the new biotechnology-derived, nutritionally improved soybean product, MON 87705, any progeny derived from crosses between MON 87705 and conventional soybean, and any progeny derived from crosses of MON 87705 with other biotechnology-derived soybean that has been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of Nutritionally Improved Soybean MON 87705

Monsanto Company has developed biotechnology-derived soybean MON 87705 with an improved fatty acid profile to enhance the suitability of soybean oil for food and industrial uses. The fatty acid (FA) levels in MON 87705 soybean oil are lower for saturated fats (6% vs. 15% FA) and higher for oleic acid (76% vs. 23%). The increase in monounsaturated fatty acid (oleic) is accompanied by an overall decrease in polyunsaturated fatty acids (17% vs 60% FA). Conventional soybean oil typically contains 60-65% polyunsaturated fatty acids (PUFA's), mostly in the form of linoleic acid. This high PUFA content makes soybean oil unsuitable for many food applications since the high concentrations of PUFA's in the oil are susceptible to oxidation and degradation at high temperature.

To improve the stability of soybean oil, the polyunsaturated fatty acids in the oil can be decreased through a process called hydrogenation that reduces the number of unstable double bonds found in fatty acids such as linolenic and linoleic, and converts them to saturated fats. Although hydrogenation produces oil with excellent thermal and oxidative stability, it also results in the production of significant levels of *trans* fatty acids in the oil. *Trans* fatty acids contribute to cardiovascular risk by elevating LDL (bad cholesterol) and reducing HDL (good cholesterol) (Kris-Etherton, 1995; Hu et al., 1997).

Soybean oil oxidative stability can be improved because the stability of vegetable oils is drastically influenced by the proportion of monounsaturates to polyunsaturates. High oleic soybean oils are estimated to have improved oxidative stability up to 17.5 times greater than conventional soybean oil (Frankel, 2005). Oil from MON 87705 would similarly have an enhanced oxidative stability (approximately three-fold) relative to

conventional soybean oil due to its increase in monounsaturated fatty acid and decrease in PUFA's, without the need for hydrogenation. Therefore, MON 87705 soybean oil can provide the food industry with options of enhanced stability for food formulation.

Numerous global health authorities recognize that diets high in total fat and saturated fat are associated with increased risk of chronic disease (FAO/WHO, 2002; Lichtenstein et al., 2006; IO, 2002; USHHS, 1988), and health experts, including the American Heart Association (Eckel et al., 2007), have recognized that an unintended consequence of a shift to oils with no or lower *trans* fat levels, may be an increase in levels of saturated fats in foods. With a fatty acid profile lower in saturated fats than commodity soybean oil, and a reduced need for hydrogenation, MON 87705 soybean oil can help address this concern as MON 87705 soybean oil contains less than 7% saturated fatty acids (palmitic + stearic acid). Based on U.S. Food and Drug Administration (FDA) guidance, a low saturated fat food contains less than 1 g of fat per serving (www.fda.gov) and the typical serving size for soybean oil is 14g (www.thumboilseed.com/soy-oil.htm). As a result, to qualify as a low saturate soybean oil the maximum amount of saturated fats allowed is 7% (0.98 g per serving). Therefore, under FDA guidance MON 87705 soybean oil can be classified as a low saturate oil.

The fatty acid profile of MON 87705 soybean oil is also well suited for industrial applications. Soybean oils have very good lubricating properties, and are highly biodegradable compared to mineral oils, but typically lack the stability needed to meet industrial requirements. Hydrogenation of soybean oil is not acceptable for most industrial uses because it leads to formation of saturated and *trans* fatty acids which can cause the oil to be solid at lower temperatures, resulting in excessive wear and tear of machinery (Kinney, 1998). The fatty acid profile of MON 87705 provides an industrial oil with improved stability that could serve as a lubricant without needing hydrogenation. In addition, soybean oil with elevated oleic acid is an attractive source for other industrial applications, such as petrochemical-derived plasticizers (Kinney, 1998). The higher oleic acid and lower saturated fat levels of MON 87705 also make it much more suitable for use in biodiesel due to its greater stability, improved cold weather performance, and reduced nitrous oxides emissions (Knothe 2005; Bringe, 2005; Graef, 2009)

As with all new biotechnology-derived traits, MON 87705 will also be bred into soybean varieties with diverse genetic backgrounds. These varieties will include commercial varieties with low linolenic acid levels which can further enhance the oxidative stability of the soybean oil. In addition, MON 87705 will be combined using traditional breeding methods with other biotechnology-derived traits, including glyphosate tolerance (MON 89788), to deliver the best agronomic platform to farmers.

To summarize, MON 87705 was developed to improve soybean oil's oxidative stability profile, without the need for hydrogenation, and lower the saturated fat content of the oil. Due to the compositional improvement in MON 87705 soybean oil, MON 87705 could expand the food market applicability of soybean oil, without contributing further to known dietary health risks or sacrificing food flavor. Similarly, MON 87705 soybean oil could also serve as an improved source for industrial and biofuel products.

I.C. Gene Suppression of *FATB* and *FAD2* and Resulting Fatty Acid Composition of the Seed

Suppression of FATB and FAD2 RNAs in soybean decreases saturated fats (16:0 palmitic acid and 18:0 stearic acid), increases oleic acid (18:1), and decreases linoleic acid (18:2).

The improved fatty acid profile in MON 87705 soybean oil is achieved through the use of endogenous soybean (*Glycine max* L.) gene segments configured to suppress *FATB* and *FAD2* gene expression. MON 87705 contains *FATB1-A* and *FAD2-1A* gene segments under the control of a seed promoter, limiting oil composition modification to this tissue. The assembled gene transcript has an inverted repeat that produces double stranded RNA (dsRNA) that, via the RNA interference (RNAi) pathway, suppresses endogenous *FATB* and *FAD2* gene expression, thereby producing the desired fatty acid phenotype. Acyl-acyl carrier protein (ACP) thioesterases (referred to herein as FATB enzymes) are localized in plastids and hydrolyze saturated fatty acids from the ACP-fatty acid moiety. The suppression of FATB results in a decrease in the transport of the saturated fats out of the plastid, thus retaining their availability for desaturation to 18:1 oleic acid (see Figure I-1). Therefore, suppression of FATB decreases saturated fat content in the oil as well as increasing oleic acid (Kinney, 1996). Subsequently, this increased amount of oleic acid is either delivered to the oil body or endoplasmic reticulum for further desaturation (Kinney, 1996). Delta-12 desaturases (referred to as FAD2 enzymes) desaturate 18:1 oleic acid to 18:2 linoleic acid. The suppression of FAD2 in soybean seed causes reduced desaturation of oleic to linoleic acid thus contributing further to the increase in oleic while reducing linoleic acid content in the oil (Dyer and Mullen, 2005). Therefore, the overall result of the suppression of these two enzymes is a reduction in saturated 16:0 palmitic and 18:0 stearic fatty acids, an increase in monounsaturated 18:1 oleic acid, and lower levels of polyunsaturated 18:2 linoleic acid relative to commodity soybean.

The RNA-based suppression of *FATB* and *FAD2* soybean genes in MON 87705 is mediated by dsRNA molecules. Double stranded RNAs are commonly found in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids. Nucleic acids have a long history of safe consumption and are considered GRAS by the U.S. Food and Drug Administration. There is no evidence to suggest dietary consumption of RNA is associated with toxicity or allergenicity. Moreover, analysis of the DNA segments encoding this dsRNA showed that they lack the sequences required for translation initiation and protein synthesis. The production of a protein from the dsRNA encoded by the insert in MON 87705 is highly unlikely. Several biotechnology-derived plant products previously deregulated by APHIS were developed using RNA-based suppression mechanisms, including virus-resistant papaya and squash, high oleic soybean, FLAVR SAVR tomatoes, and plum trees resistant to Plum pox virus (FDA, 1994; USDA-APHIS, 1994; USDA-APHIS, 1997; USDA-APHIS, 2006; USDA-APHIS, 2007). Based on this information, it is concluded that the inserted DNA and resulting dsRNA are safe and unlikely to produce a protein or polypeptide. As a result, the RNA-based suppression technology used in MON 87705 poses no novel risks from a food, feed or environmental perspective.

To examine suppression of the RNA levels of the endogenous *FAD2-1A* and *FATB1-A* genes, immature seed[2] at stage R5/6 were subjected to northern blot analyses and compared to a conventional control[3] that has a genetic background similar to the test substance. The details of the materials and methods are described in Appendix J.

To confirm the outcome of gene suppression on the seed fatty acid profile of MON 87705, fatty acid analysis was performed on MON 87705 compared to a conventional control. This analysis indicates the impact of the suppression of *FATB* and FAD2 genes on fatty acid profiles. The details of the full compositional analyses of MON 87705 are in Section VII, and the materials and methods for these analyses are described in Appendix E from Section VII.

Northern blot analysis of FAD2-1A RNA level in MON 87705 confirms suppression.

PolyA enriched RNA (PolyA+ RNA) isolated from approximately 50 µg total RNA from immature seed was resolved on a formaldehyde/agarose gel, blotted, and hybridized to the FAD2-1A probe (Figure I-2A). Approximately 200 pg of the FAD2-1A probe template was loaded on the gel to serve as a positive control, and the probe template produced a hybridization signal at approximately 0.4 kb (Figure I-2A, lane 9). Approximately 50 pg of actin probe template was loaded on the gel to serve as a negative control and, as expected, there is no hybridization signal produced (Figure I-2A, lane 10). The detection of the probe template hybridization control demonstrates that the probe is hybridizing only to the target DNA sequence.

PolyA+ RNA from each of four replicates of the conventional soybean control immature seeds (Figure I-2A, lanes 1, 3, 5, and 7) produced a strong hybridization signal at approximately 1.5 kb which is the expected size of the *FAD2-1A* transcript, whereas polyA+ RNA isolated from each of the four replicates of MON 87705 immature seeds (Figure I-2A, lanes 2, 4, 6, and 8) produced a very faint hybridization signal of approximately 1.5 kb at a greatly reduced level relative to the conventional control RNA. There were faint, nonspecific hybridization signals observed at approximately 1.7 kb, 3.2 kb, and 3.6 kb, which were detected in both the conventional soybean control and MON 87705. These data show a reduction in the levels of detectable *FAD2-1A* RNA in MON 87705 compared to conventional soybean.

In order to confirm that the reduced *FAD2-1A* RNA levels in MON 87705 was not due to a reduced RNA loading on the gel or poor RNA quality, the FAD2-1A hybridization signal was removed and the blot was re-hybridized with the actin probe (Figure I-2B). The approximately 50 pg of actin probe template that was loaded on the gel as a positive hybridization control showed a band at approximately 0.9 kb (Figure I-2B, lane 10). There is a faint band at approximately 1.8 kb, which most likely resulted from dimerization of the actin probe template. In lane 9, there is a band at approximately 0.4 kb, which likely results from the incomplete removal of the FAD2-1A probe template.

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[2] For studies presented throughout this petition referring to the use of MON 87705, conventional control, or reference material seed, the term "seed" refers to the harvested seed.

[3] For studies presented throughout this petition comparing MON 87705 to the conventional control, the conventional control was a nontransgenic parental soybean with genetics similar to MON 87705, but lacked the introduced traits

There is no hybridization signal with the actin probe at approximately 0.9 kb, as expected from a negative control (Figure I-2B, lane 9). The detection of the actin probe template hybridization control demonstrates that the probe is hybridizing only to the target DNA sequence.

PolyA+ RNA from conventional (Figure I-2B, lanes 1, 3, 5, and 7) and MON 87705 immature seeds (Figure I-2B, lanes 2, 4, 6, and 8) showed a strong hybridization signal at approximately 1.6 kb, which is expected for the actin transcript. The hybridization signals from conventional soybean and MON 87705 immature seeds have similar intensities, indicating that the RNA loading, RNA quality, and hybridization between conventional and MON 87705 are similar. Therefore, the difference in the FAD2-1A hybridization signals between conventional and MON 87705 reflects a large decrease in *FAD2-1A* RNA level.

Northern blot analysis of FATB1-A RNA level in MON 87705 confirms suppression.

PolyA+ RNA isolated from approximately 100 µg total RNA from immature seed was resolved on a formaldehyde/agarose gel, blotted, and hybridized to the FATB1-A probe (Figure I-3A). Approximately 10 pg of the FATB1-A probe template was loaded on the gel to serve as a positive control. As expected, the probe template produced a hybridization signal at approximately 0.4 kb (Figure I-3A, lane 9). Approximately 50 pg of the actin probe template was loaded on the gel to serve as a negative control and, as expected, there is no signal produced (Figure I-3A, lane10). The detection of the FATB1-A probe template hybridization control demonstrates that the probe is hybridizing only to the target DNA sequence.

PolyA+ RNA from four replicates of the immature seeds from conventional soybean (Figure I-3A, lanes 1, 3, 5, and 7) produced a strong hybridization signal at approximately 1.8 kb, which is expected for the *FATB1-A* transcript, whereas polyA+ RNA isolated from four replicates of MON 87705 immature seeds (Figure I-3A, lanes 2, 4, 6, and 8) also produced a hybridization signal of approximately 1.8 kb, but at a much reduced level. In addition, there was a hybridization signal at approximately 1.5 kb, which was not present in the conventional soybean control. The approximately 1.5 kb signal is likely a degraded *FATB1-A* transcript. These data indicate a reduction in the RNA levels of the *FATB1-A* in MON 87705 compared to conventional soybean.

In order to confirm that the difference in the hybridization of the FATB1-A probe in MON 87705 is not due to a reduced RNA loading on the gel or poor RNA quality, the FATB1-A hybridization signal was removed and the blot re-hybridized with the actin probe after stripping (Figure I-3B). The approximately 50 pg of actin probe template that was loaded on the gel as a positive control showed a band at approximately 0.9 kb (Figure I-3B, lane 10). The FATB1-A probe template did not produce any signal, as expected from a negative control (Figure I-3B, lane 9). The detection of the actin probe template control demonstrates that the probe is hybridizing only to the target DNA sequence.

PolyA+ RNA from the conventional soybean control (Figure I-3B, lanes 1, 3, 5, and 7) and MON 87705 immature seed (Figure I-3B, lanes 2, 4, 6, and 8) showed a strong hybridization band at approximately 1.6 kb, which is expected for the actin transcript and

the intensity of the bands are similar between the conventional control and MON 87705. These data indicate that the RNA loading, RNA quality, and hybridization between conventional soybean and MON 87705 are similar. Therefore the difference in the FATB1-A hybridization signals between conventional and MON 87705 reflects the difference in *FATB1-A* RNA level.

Fatty Acid Composition of MON 87705shows predicted phenotype.

An assessment of the seed fatty acid profile collected from five field sites in Chile during 2007/2008 demonstrates MON 87705 has the intended fatty acid changes. MON 87705 had a decrease in saturated fats (from 15.33% to 5.67% FA: 16:0 palmitic acid plus 18:0 stearic acid), an increase in oleic acid (from 22.81% to 76.47% FA), and a decrease in linoleic acid (from 52.86% to 10.10% FA). Statistically significant differences reflecting intended seed fatty acid changes were observed in the combined-site analysis (Table I-1) and at each individual site (Appendix E).

Conclusion

The suppression of *FATB* in soybean seed decreases both of the major saturated fatty acids (16:0 palmitic acid and 18:0 stearic acid), and suppression of *FAD2* in soybean seeds increases oleic acid (18:1) and subsequently decreases linoleic acid (18:2) in soybean oil (Dyer and Mullen, 2005). MON 87705 northern blot data confirms the suppression of endogenous *FAD2-1A* and *FATB1-A* RNAs. MON 87705 seed composition data demonstrates that suppression of these endogenous RNAs produces the intended alteration in fatty acid profile, which is a lower level of saturated fats (16:0 palmitic acid and 18:0 stearic acid), a higher level of oleic acid (18:1), and an associated lower level of linoleic acid (18:2).

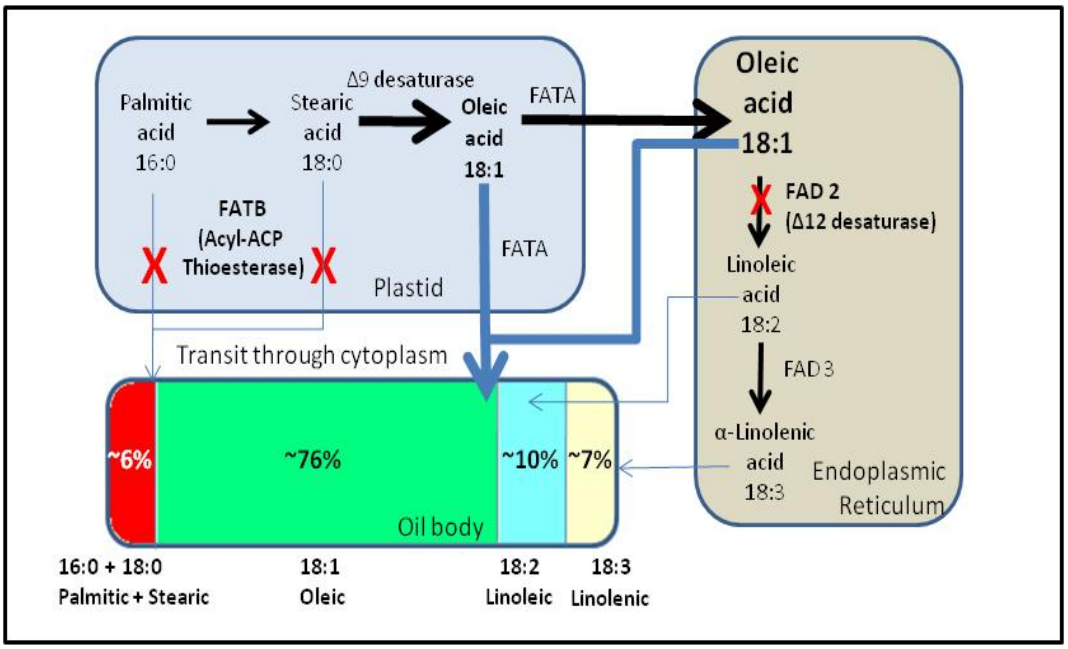


Figure I-1. Schematic of the Soybean Fatty Acid Biosynthetic Pathway
✕ indicates suppression of endogenous *FATB* and *FAD2* in MON 87705 seeds

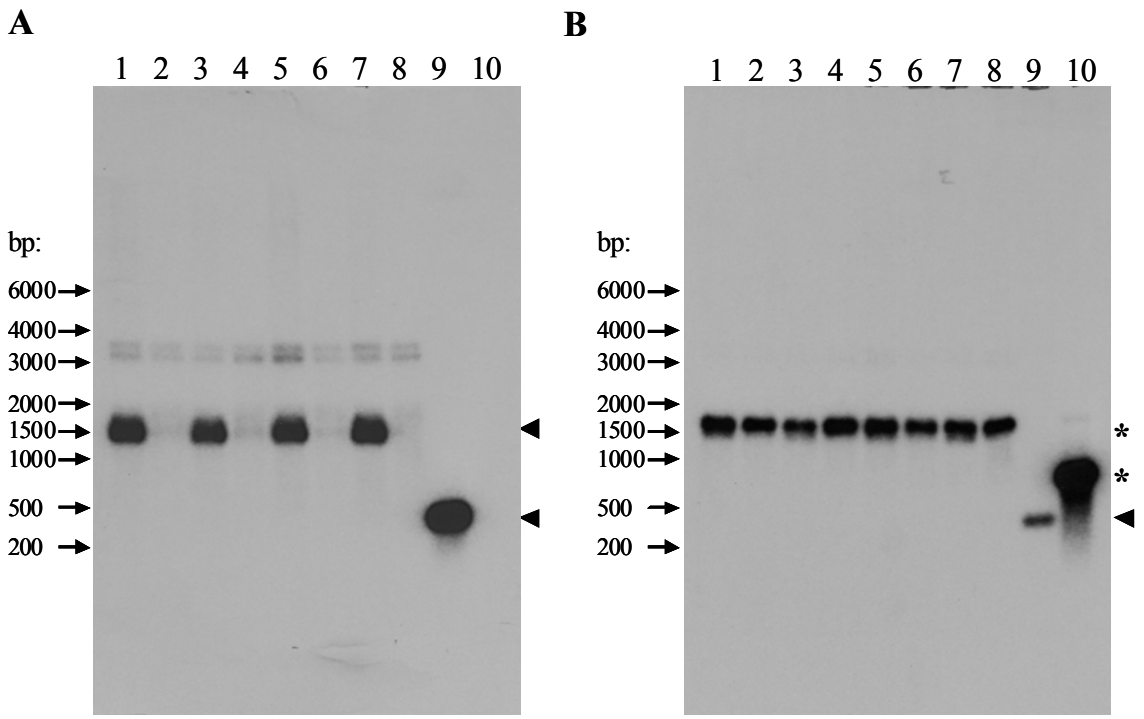


Figure I-2. Northern Blot Analysis of *FAD2-1A* RNA Level in MON 87705
Panel A and Panel B are the same northern blot containing polyA+ RNA isolated from stage R5/6 immature seed tissue. Panel A is hybridized with the FAD2-1A probe. Panel B is hybridized with the actin probe after removal of the FAD2-1A signal. Arrow heads indicate FAD2-1A hybridization signals and stars indicate the actin hybridization signal. Lane designations are as follows:

Lane 1: Conventional control (PolyA+ RNA replicate 1)
2: MON 87705 (PolyA+ RNA replicate 1)
3: Conventional control (PolyA+ RNA replicate 2)
4: MON 87705 (PolyA+ RNA replicate 2)
5: Conventional control (PolyA+ RNA replicate 3)
6: MON 87705 (PolyA+ RNA replicate 3)
7: Conventional control (PolyA+ RNA replicate 4)
8: MON 87705 (PolyA+ RNA replicate 4)
9: FAD2-1A probe template (200 pg)
10: Actin probe template (50 pg)

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

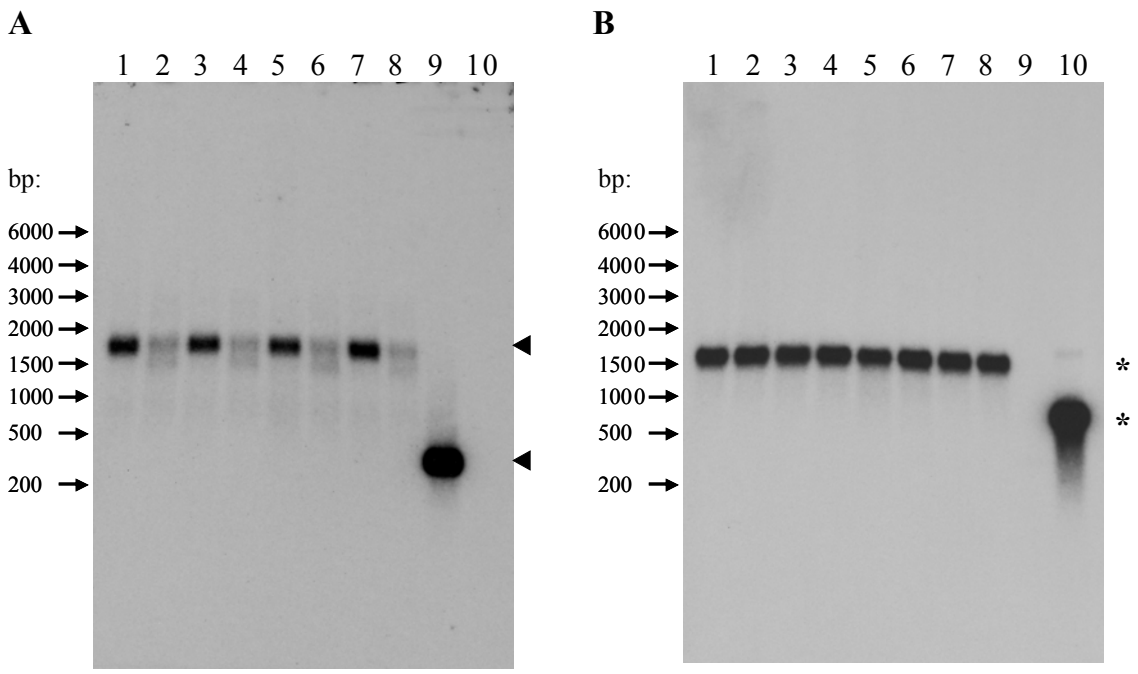


Figure I-3. Northern Blot Analysis of *FATB1-A* RNA Level in MON 87705
Panel A and Panel B is the same northern blot containing polyA+ RNA isolated from stage R5/6 immature seed tissue. Panel A is hybridized with the FATB1-A probe. Panel B is hybridized with the actin probe after removal of the FATB1-A signal. Arrow heads indicate FATB1-A hybridization signals and stars indicate the actin hybridization signal. Lane designations are as follows:

Lane 1: Conventional control (PolyA+ RNA replicate 1)
2: MON 87705 (PolyA+ RNA replicate 1)
3: Conventional control (PolyA+ RNA replicate 2)
4: MON 87705 (PolyA+ RNA replicate 2)
5: Conventional control (PolyA+ RNA replicate 3)
6: MON 87705 (PolyA+ RNA replicate 3)
7: Conventional control (PolyA+ RNA replicate 4)
8: MON 87705 (PolyA+ RNA replicate 4)
9: FATB1-A probe template (10 pg)
10: Actin probe template (50 pg)

→ Symbol denotes size of RNA, in base pairs, obtained from MW markers on ethidium stained gel

Table I-1. Summary of Product Concept Fatty Acid Levels for Test (MON 87705) vs. the Conventional Control (A3525) and Commercial Tolerance Interval

Component (Units)	MON 87705 Mean [Range]	A3525 Mean [Range]	Commercial Tolerance Interval[1]
Statistical Differences Observed in Combined-Site Analysis Seed Fatty Acid (% Total FA)			
16:0 Palmitic	2.36 [2.25 - 2.44]	10.83 [10.51 – 11.08]	[7.62, 12.55]
18:0 Stearic	3.31 [3.07 - 3.82]	4.50 [4.24 – 4.85]	[2.87, 7.15]
18:1 Oleic	76.47 [73.13 - 79.17]	22.81 [21.41 – 25.08]	[18.40, 30.22]
18:2 Linoleic	10.10 [7.85 - 12.42]	52.86 [51.68 – 53.89]	[47.75, 56.46]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties

I.D. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of biotechnology-derived crops that do not include plant-incorporated protectants falls on two federal agencies: FDA and USDA. Deregulation of MON 87705 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87705 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions.

Submission to FDA

MON 87705 falls within the scope of the 1992 U.S. Food and Drug Administration's (FDA) policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (FDA, 1992). In compliance with this policy, Monsanto will initiate a consultation with the FDA on the food and feed safety and nutritional assessment summary for MON 87705.

Submissions to Foreign Government Agencies

To support commercial introduction of MON 87705 in the U.S., regulatory submissions will be made to countries that import significant quantities of soybean or its processed fractions from the U.S. and have established regulatory approval processes in place. These will include submissions to a number of foreign government regulatory authorities,

including: Ministry of Agriculture, People's Republic of China; Japan's Ministry of Agriculture, Forestry, and Fisheries (MAFF) and the Ministry of Health, Labor, and Welfare (MHLW); the Canadian Food Inspection Agency (CFIA) and Health Canada; the Intersectoral Commission for Biosafety of Genetically Modified Organisms (CIBIOGEM), Mexico; 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.

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), and 2) a summary prepared by USDA-APHIS (2006) and a biology document published by Canadian Food Inspection Agency-Plant Biosafety Office (CFIA, 1996).

II.A. Soybean as a Crop

Soybean is the most prevalently grown oilseed in the world, with approximately 222.1 million metric tons (MMT) of harvested seed produced in 2007, which represented 56% of world oilseed seed production that year (ASA, 2008; Soyatech, 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 (Soyatech, 2008); also see Table II 1. Approximately one-third of the 2007 world soybean production was produced in the U.S. (Soyatech, 2008). The soybean produced in China and India are primarily for domestic use, while a significant portion of soybean 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; Soyatech, 2008).

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 (Soyatech, 2008).

Approximately 50% of the world soybean seed supply was crushed to produce soybean meal and oil in 2007 (ASA, 2008; Soyatech, 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 (Soyatech, 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 (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 to 2007/08 (Soyatech, 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.

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 only could 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 cultivated extensively and improved through conventional breeding following its introduction in the U.S. and subsequently has become a key source of nutrients for food and feed use in the U.S. (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.

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

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

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

Glycine soja grows wild in China, Japan, Korea, the Russian Far East, and Taiwan, and is commonly found in fields, hedgerows, roadsides, and riverbanks (Lu, 2004). The plant is

an annual, slender in build with narrow trifoliolate leaves. The purple or very rarely white flowers are inserted on short, slender racemes. The pods are short and tawny with hirsute pubescence, producing oval-oblong seeds (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 subspherical 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).

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 adjacent plants of other soybean varieties

occurring at very low frequency (0 to 6.3%) in adjacent plants (Caviness, 1966; Ray et al., 2003; Yoshimura et al., 2006). Pollination typically takes place on the day the flower opens. The pollen naturally comes in contact with the stigma during the process of anthesis. Anthesis normally occurs in late morning, depending on the environmental conditions. The pollen usually remains viable for two to four hours, and no viable pollen can be detected by late afternoon. Natural or artificial cross-pollination can only take place during the short time when the pollen is viable.

II.F. Cultivated Soybean as a Volunteer

Cultivated soybean plants are annuals, and they reproduce solely by means of seeds. Volunteer soybean in rotational crops is typically not a concern in most environments where soybean is cultivated (CFIA, 1996; OECD, 2000). Soybean seed rarely exhibit any dormancy characteristics, and seed remaining in the field after harvest likely will readily imbibe water (Lersten and Carlson, 2004), germinate, and will be killed by frost or field cultivation. If 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 87705 was A3525, a nontransgenic conventional variety developed by Asgrow Seed Company. A3525 is a mid-maturity Group III soybean variety with very high yield potential. It has superior yields relative to lines of similar maturity and has excellent agronomic characteristics (Steffen, 2004).

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

In developing the data to support the safety assessment of MON 87705, A3525 was used as the nontransgenic conventional soybean comparator. In general, the genetic background of MON 87705 was matched with that of the control so the effect of the genetic insertion and the presence of the CP4 EPSPS protein could be assessed in an unbiased manner. Since MON 87705 was derived from the A3525 conventional variety, it was deemed appropriate to use the nontransformed A3525 as the control variety because 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. The reference varieties used at each location were selected based on their availability and agronomic fit (Appendix F and Table F-1).

III. DESCRIPTION OF THE TRANSFORMATION SYSTEM

MON 87705 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue using the double-border, binary vector PV-GMPQ/HT4404 (Section IV, Figure IV-1). The vector, PV-GMPQ/HT4404, contains both the left and right border sequences flanking the transfer DNA (T-DNA) to facilitate transformation.

This vector, PV-GMPQ/HT4404, is approximately 13.1 kb and contains two T-DNAs, each delineated by left and right border regions. The first T-DNA, designated T-DNA I, contains a *cp4 epsps* expression cassette and a partial suppression cassette. The *cp4 epsps* expression cassette is under the regulation of *FMV/Tsf1* chimeric promoter and *E9* polyadenylation sequence. The partial suppression cassette in T-DNA I contains the sense segments of the *FAD2-1A* intron and *FATB1-A* 5' UTR, including the chloroplast targeting sequence, that are under the regulation of the seed *7Sα*' promoter. The second T-DNA, designated T-DNA II, contains a partial suppression cassette that consists of the antisense segments of *FAD2-1A* intron and *FATB1-A* 5′ UTR, which is flanked by the *H6* untranslated sequence. During plant transformation, the two T-DNAs co-integrated at one locus in the soybean genome, creating a DNA insert that contains a *cp4 epsps* cassette and a single *FAD2-1A* and *FATB1-A* suppression cassette.

The *Agrobacterium*-mediated soybean transformation to produce MON 87705 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 A3525 seed. After co-culturing with *Agrobacterium* carrying the vector, the meristems were placed on selection medium containing glyphosate to inhibit the growth of untransformed plant cells, as well as carbenicillin and Claforan to inhibit excess *Agrobacterium*. The meristems were then placed in media conducive to shoot and root development, and only rooted plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

The R_0 plants generated through this transformation were self-pollinated, and the subsequent R_1 plants were screened for the zygosity of inserted gene. Only the R_1 plants that were homozygous for the insertion, as determined by quantitative PCR, and produced seeds with the desired fatty acid composition were advanced for development. Their progeny were subjected to further phenotypic assessments. MON 87705 was selected as the lead event based on superior phenotypic characteristics, agronomics, and molecular profile. Regulatory studies on MON 87705 were initiated to further characterize the genetic insertion and the expressed protein, and to establish the food, feed, and environmental safety relative to conventional soybean. The major steps involving the development of MON 87705 are depicted in Figure III-1.

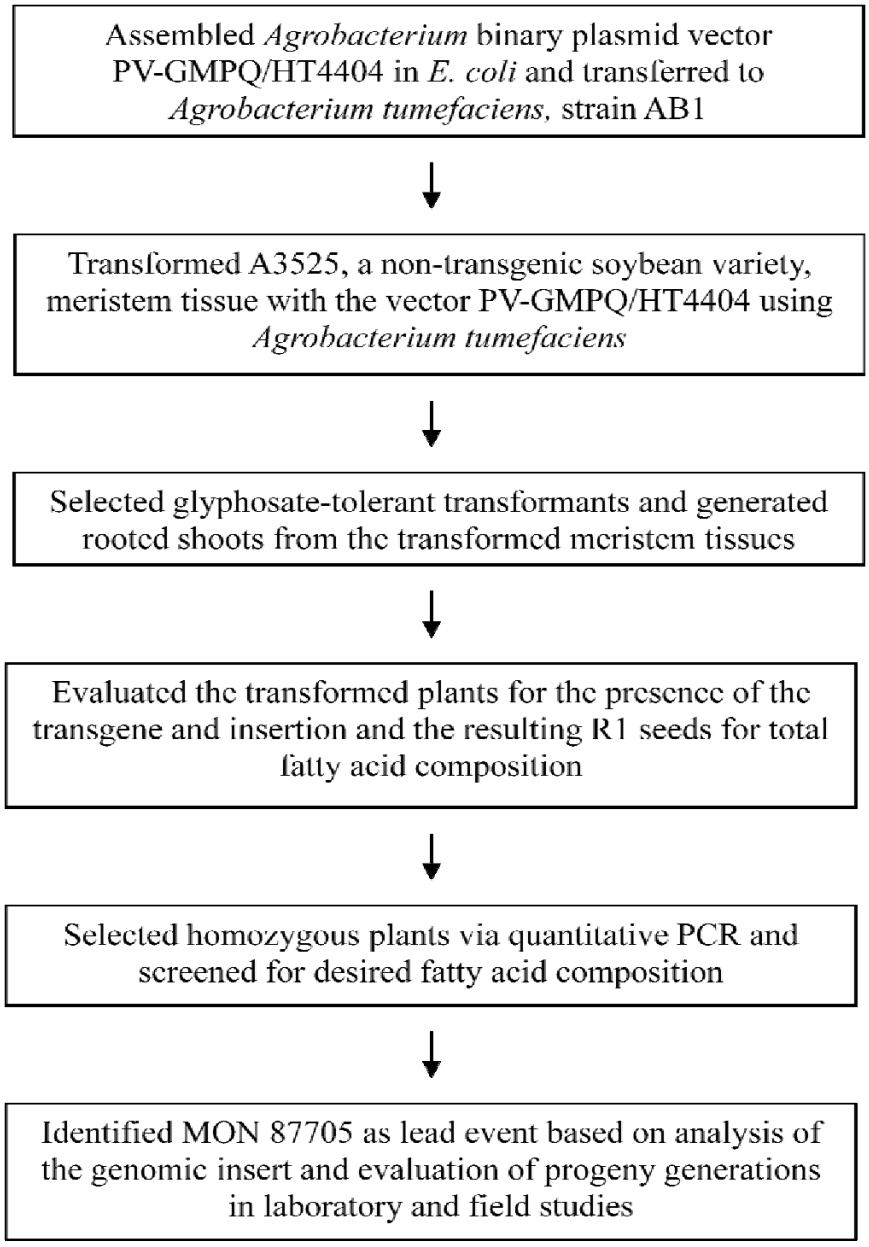


Figure III-1. Schematic of the Development of MON 87705

IV. GENETIC ELEMENTS

This section describes the vector, the donor genes and the regulatory elements used in the development of MON 87705 and the deduced amino acid sequence of the CP4 EPSPS protein produced in MON 87705. In this section, T-DNA refers to DNA that is transferred to the plant during transformation. An expression cassette is comprised of sequences to be transcribed and the regulatory elements necessary for the expression of those sequences. The suppression cassette refers to the sequences and regulatory elements necessary for the suppression of the endogenous *FAD2* and *FATB* RNA transcripts.

IV.A. Vector GMPQ/HT4404

The PV-GMPQ/HT4404 vector used for the transformation of soybean to produce MON 87705 is shown in Figure IV-1 and its elements described in Table IV-1. This vector is approximately 13.1 kb and contains two T-DNAs, each delineated by left and right border regions. T-DNA I contains a *cp4 epsps* expression cassette and a partial suppression cassette. The *cp4 epsps* expression cassette is under the regulation of *FMV/Tsf1* chimeric promoter and *E9* polyadenylation sequence. The partial suppression cassette in T-DNA I contains the sense segments of the *FAD2-1A* intron and *FATB1-A* 5' UTR, including the chloroplast targeting sequence, which are under the regulation of the seed *7Sα'* promoter. T-DNA II contains a partial suppression cassette, which consists of the antisense segment of *FAD2-1A* intron and *FATB1-A* 5′ UTR that is flanked by the *H6* untranslated sequence. During plant transformation, a portion of the plants that were generated contained the two T-DNAs co-integrated at one locus in the soybean genome creating a DNA insert that contains a *cp4 epsps* cassette and a single *FAD2-1A* and *FATB1-A* suppression cassette.

The vector backbone region outside of the T-DNA contains two origins of replication for maintenance of plasmid in bacteria (*OR-oriV*, *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-GMPQ/HT4404 is provided in Table IV-1.

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

The *cp4 epsps* gene expression cassette is present in MON 87705. The *cp4 epsps* coding sequence encodes a 47.6 kDa CP4 EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996b). The *cp4 epsps* gene expression cassette was used as a selectable marker during the transformation to produce MON 87705. The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup herbicides, relative to endogenous plant EPSPS (Padgette et al., 1996a). The CP4 EPSPS protein confers resistance 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 protein full-length amino acid sequence is shown in Figure IV-3.

IV.C. The *FAD2-1A* and *FATB1-A* segments Sequence (T-DNA I and II)

MON 87705 contains a partial sequence of the soybean (*Glycine max*) *FAD2-1A* gene and *FATB1-A* gene. The *FAD2-1A* and *FATB1-A* gene segments are comprised of ~ 0.6 kb of sequence from the *FAD2-1A* intron and the *FATB1-A* 5′ UTR, and form the MON 87705 suppression cassette. This suppression cassette expresses an inverted repeat of the *FAD2-1A* and *FATB1-A* gene segments. The assembled gene transcript has an inverted repeat that produces double stranded RNA (dsRNA) that, via the RNA interference (RNAi) pathway, suppresses endogenous *FATB* and *FAD2* RNA, and, ultimately, an improved fatty acid composition in the seed.

IV.D. Regulatory Sequences

The *cp4 epsps* coding sequence that is located in T-DNA I, is under the regulation of the *FMV/Tsf1* promoter, the *Tsf1* leader and intron, the CTP2 targeting sequence and the *E9* 3' untranslated sequence. The *FMV/Tsf1* is a chimeric promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic virus 35S RNA (Richins et al., 1987) combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989). The *Tsf1* leader is the 5' untranslated region from the *Arabidopsis thaliana Tsf1* gene (Axelos et al, 1989) that encodes the elongation factor EF-1 alpha. 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* 3′ untranslated region is the 3' untranslated sequence from the *RbcS2* gene of *Pisum sativum* (Coruzzi et al., 1984) and is present to direct polyadenylation of the *cp4 epsps* transcript.

Also located in T-DNA I is a partial suppression cassette with a portion of the *FAD2-1A* intron and a portion of the *FATB1-A* 5' UTR, including the plastid targeting sequence under the regulation of the *Glycine max 7Sα′* seed storage gene promoter (designated *Sphas* 1) which drives expression in immature seeds (Doyle et al., 1986).

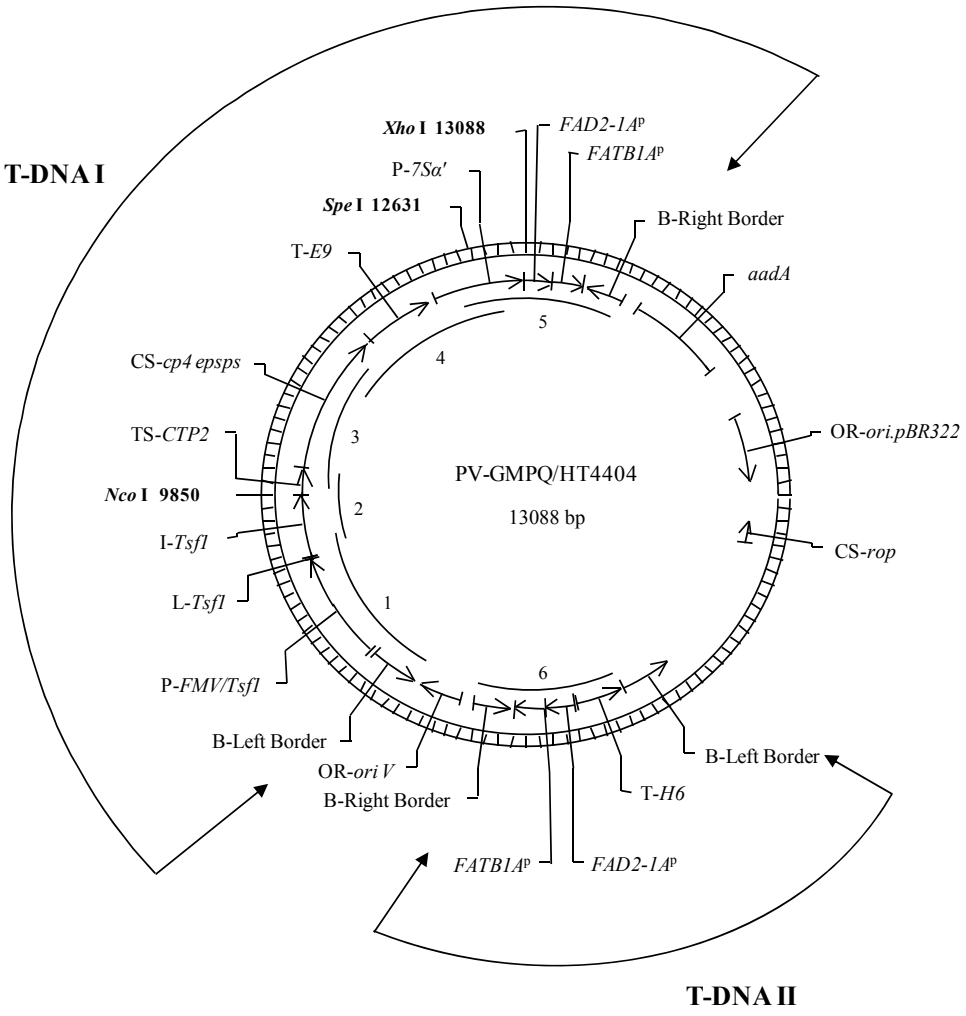
T-DNA II contains another partial suppression cassette with the *FAD2-1A* and *FATB1-A* gene segments followed by the *H6* 3' untranslated sequence. The *H6* 3' untranslated region is from the *Gossypium barbadense* cotton fiber gene and is present to terminate transcription (John and Keller, 1995).

IV.E. T-DNA Borders

Plasmid PV-GMPQ/HT4404 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; Zambryski et al., 1982). The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation. The border regions delineate the T-DNA and are involved in their efficient transfer into the soybean genome. Because PV-GMPQ/HT4404 is a two T-DNA vector, it contains two right border regions and two left border regions, where one set flank T-DNA I and the other set flank T-DNA II (see description above).

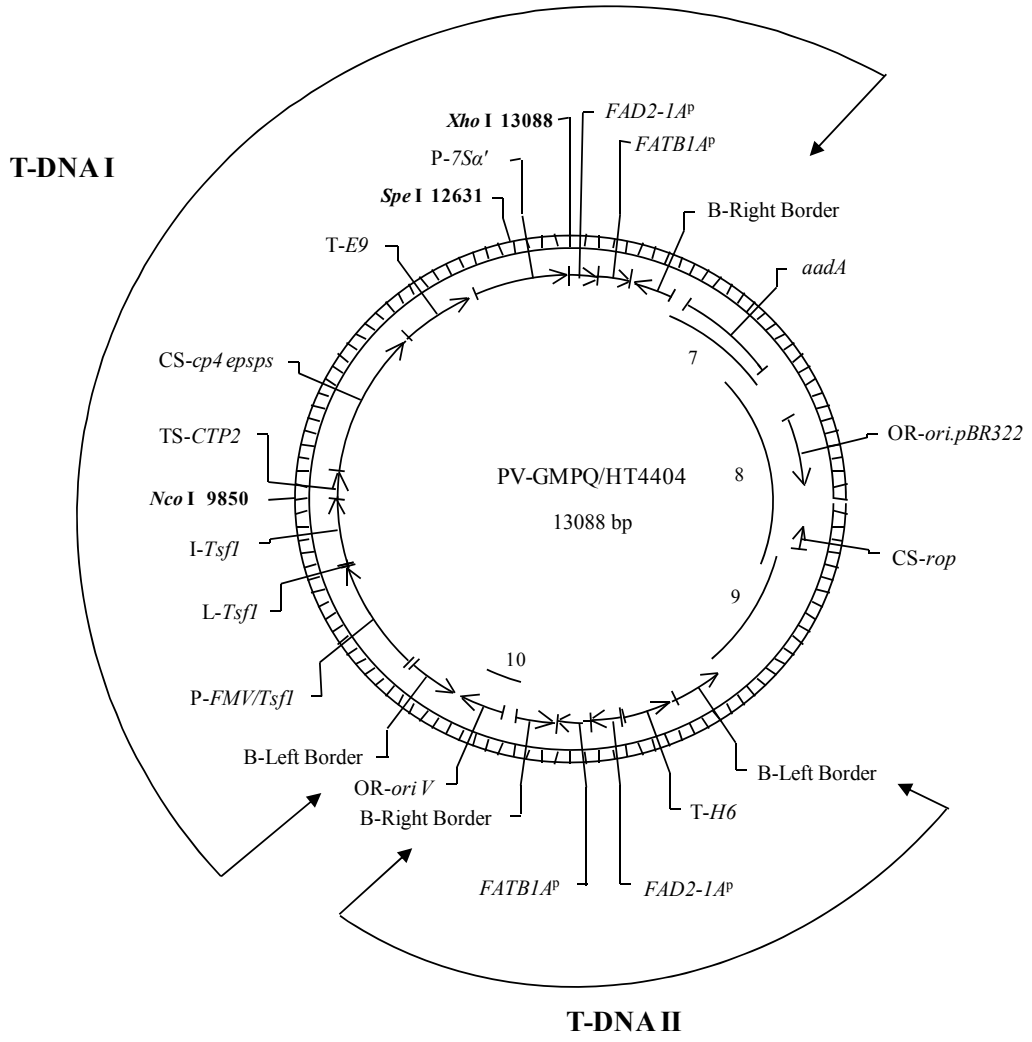
IV.F. Genetic Elements Outside of the T-DNA Borders

Genetic elements that exist outside of the T-DNA borders are those that are essential for the maintenance and selection of the vector PV-GMPQ/HT4404 in bacteria. The origin of replication *OR-ori V* is required for the maintenance of the plasmid in *Agrobacterium* (Stalker et al., 1981) and is derived from the broad host plasmid RK2. The origin of replication OR-*ori.pBR322* is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid pBR322 (Sutcliffe, 1979). 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. Because these elements are outside the border regions, they are not expected to be transferred into the soybean genome. The absence of the backbone sequence in MON 87705 has been confirmed by Southern blot analyses (see Section V.B.).



Probe	DNA Probe	Start Position	Stop Position	Total Length (~kb)
1	T-DNA I Probe 1A	7657	9406	1.8
2	T-DNA I Probe 2B	9270	10042	0.8
3	T-DNA I Probe 3C	9943	11325	1.4
4	T-DNA I Probe 4D	11151	160	2.1
5	T-DNA I Probe 5E	13080	973	1.0
6	T-DNA II Probe 1A	5570	6693	1.1

Figure IV-1. Circular Map of Plasmid PV-GMPQ/HT4404 showing probes 1-6
Plasmid PV-GMPQ/HT4404 containing the T-DNAs used in *Agrobacterium*-mediated transformation to produce MON 87705. Genetic elements (depicted in the exterior of the map) and restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The T-DNA I and T-DNA II probes used in the Southern analyses (labeled 1-6 within the interior of the map) are detailed in the accompanying table.



Probe	DNA Probe	Start Position	Stop Position	Total Length (~kb)
7	Backbone Probe 1	974	2280	1.3
8	Backbone Probe 2	2140	4080	1.9
9	Backbone Probe 3	3631	5127	1.5
10	Backbone Probe 4	7025	7656	0.6

Figure IV-2 Plasmid Map of Vector PV-GMPQ/HT4404 Showing Probes 7-10
Plasmid PV-GMPQ/HT4404 containing the T-DNAs used in *Agrobacterium*-mediated transformation to produce MON 87705. Genetic elements (depicted in the exterior of the map) and restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The backbone probes used in the Southern analyses (labeled 7-10 within the interior of the map) are detailed in the accompanying table.

Table IV-1. Summary of Genetic Elements in Plasmid Vector PV- GMPQ/HT4404

Genetic Element	Location in Plasmid	Function (Reference)
T-DNA I		
B[1]-Left Border	7657 – 8098	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Intervening Sequence	8099 – 8134	Sequence used in DNA cloning
P[2]-*FMV/Tsf1*	8135 – 9174	Chimeric promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic virus 35S RNA (Richins et al., 1987) combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1alpha (Axelos et al., 1989)
L[3]-*Tsf1*	9175 – 9220	5' untranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
I[4]-*Tsf1*	9221 – 9842	Intron with flanking exon sequence from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1alpha (Axelos et al., 1989)
Intervening Sequence	9843 – 9851	Sequence used in DNA cloning
TS[5]-*CTP2*	9852 – 10079	Targeting sequence from the *ShkG* gene encoding the transit peptide region of *Arabidopsis thaliana* EPSPS (Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS[6]-*cp4 epsps*	10080 –11447	Codon modified coding sequence of the *aroA* gene from the *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgette et al., 1996a)
Intervening Sequence	11448 – 11505	Sequence used in DNA cloning
T[7]-*E9*	11506 – 12148	3′ untranslated region of the pea *RbcS2* gene which functions to direct polyadenylation of the mRNA (Coruzzi et al., 1984)
Intervening Sequence	12149 – 12236	Sequence used in DNA cloning
P-*7Sα′*	12237 – 13077	Promoter and leader from the Sphas1 gene of *Glycine max* encoding beta-conglycinin storage protein (alpha'-bcsp) (Doyle et al., 1986) that directs transcription in seed

B[1] – Border; **P[2]** – Promoter; **L[3]**– Leader; **I[4]** – Intron; **TS[5]** – Targeting Sequence; **CS[6]** – Coding Sequence;
T[7] – 3' untranslated transcriptional termination sequence and polyadenylation signal sequences;

Table IV-1 (cont.). Summary of Genetic Elements in Plasmid Vector PV-GMPQ/HT4404

Intervening Sequence	13078 – 11	Sequence used in DNA cloning
***FAD2-1A*^{p}**	12 – 277	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
***FATB1-A*^{p}**	278 – 578	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	579 – 616	Sequence used in DNA cloning
B-Right Border	617 – 973	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Zambryski et al., 1982)
Vector Backbone		
Intervening Sequence	974 – 1109	Sequence used in DNA cloning
aadA	1110 – 1998	Promoter, coding sequence, and 3' UTR for an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase from the transposon Tn7 (Fling et al., 1985) that confers spectinomycin and streptomycin resistance
Intervening Sequence	1999 – 2528	Sequence used in DNA cloning
OR^{8}-*ori-pBR322*	2529 – 3117	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)
Intervening Sequence	3118 – 3544	Sequence used in DNA cloning
CS-*rop*	3545 – 3736	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening Sequence	3737 – 5127	Sequence used in DNA cloning
T-DNA II		
B-Left Border	5128 – 5569	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)

^{P} – Partial sequence; OR^{8} – Origin of Replication.

Table IV-1 (cont.). Summary of Genetic Elements in Plasmid Vector PV-GMPQ/HT4404

Intervening Sequence	5570 – 5667	Sequence used in DNA cloning
T-*H6*	5668 – 6103	3' UTR sequence of the *H6* gene from *Gossypium barbadense* encoding a fiber protein involved in secondary cell wall assembly (John and Keller, 1995)
Intervening Sequence	6104 – 6115	Sequence used in DNA cloning
FAD2-1A^p	6116 – 6381	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
FATB1-A^p	6382 – 6682	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	6683 – 6693	Sequence used in DNA cloning
B-Right Border	6694 – 7024	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Zambryski et al., 1982)
Vector Backbone		
Intervening Sequence	7025 – 7173	Sequence used in DNA cloning
OR-*ori V*	7174 – 7570	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981).
Intervening Sequence	7571 – 7656	Sequence used in DNA cloning

```
     MLHG ASSRPATARK SSGLSGTVRI PGDKSISHRS FMFGGLASGE TRITGLLEGE DVINTGKAMQ AMGARIRKEG
DTWIIDGVGN GGLLAPEAPL DFGNAATGCR LTMGLVGVYD FDSTFIGDAS LTKRPMGRVL NPLREMGVQV KSEDGDRLPV
TLRGPKTPTP ITYRVPMASA QVKSAVLLAG LNTPGITTVI EPIMTRDHTE KMLQGFGANL TVETDADGVR TIRLEGRGKL
TGQVIDVPGD PSSTAFPLVA ALLVPGSDVT ILNVLMNPTR TGLILTLQEM GADIEVINPR LAGGEDVADL RVRSSTLKGV
TVPEDRAPSM IDEYPILAVA AAFAEGATVM NGLEELRVKE SDRLSAVANG LKLNGVDCDE GETSLVVRGR PDGKGLGNAS
GAAVATHLDH RIAMSFLVMG LVSENPVTVD DATMIATSFP EFMDLMAGLG AKIELSDTKA A
```

Figure IV-3. Deduced Amino Acid Sequence of the Mature CP4 EPSPS Protein in PV-GMPQ/HT4404

The amino acid sequence of the CP4 EPSPS protein was deduced from the full-length coding nucleotide sequence present in vector PV-GMPV/HT4404.

V. GENETIC ANALYSIS

A multi-faceted approach was taken to characterize the genetic modification made to produce MON 87705. The results confirm that MON 87705 contains a single insert that is stably integrated and is inherited according to Mendelian principles over multiple generations. These conclusions were based on several lines of evidence: 1) Southern blot analyses to assay the entire soybean genome for the presence of DNA derived from the transformation plasmid PV-GMPQ/HT4404, and to confirm that a single copy of the *cp4 epsps* expression cassette and the *FAD2-1A* and *FATB1-A* suppression cassette was inserted at a single site and that the insert is stably inherited; 2) DNA sequencing analyses to determine the exact sequence of the inserted DNA and allow a comparison to the T-DNA sequence in the transformation vector to confirm that only the expected sequences were integrated; and 3) a comparison of the genomic DNA flanking the insert to the sequence of the insertion site in conventional soybean to identify any rearrangements that occurred at the insertion site during transformation. Taken together, the characterization of the genetic modification demonstrates that a single copy of the T-DNA was inserted at a single locus of the genome.

Southern blot analyses were used to determine the number of copies and the insertion sites of the integrated DNA as well as the presence or absence of plasmid backbone sequence. The Southern blot strategy was designed to ensure that all potential transgenic segments would have been identified. The entire soybean genome was assayed with probes that spanned the complete transformation plasmid to detect the presence of the insertion as well as confirm the absence of any backbone sequence. This was accomplished by using probes that were less than 2 kb in length ensuring a high level of sensitivity. This high level of sensitivity was demonstrated for each blot by detection of a positive control added at the equivalent of $1/10^{th}$ of a genome. Two restriction enzyme sets were specifically chosen to independently confirm the presence of the insert. This two enzyme design also maximizes the possibility of detecting an insertion elsewhere in the genome which could be missed if that band comigrated with an expected band. Additionally, the restriction enzyme sets were chosen such that at least one enzyme from each set resides in the known 5′ or 3′ flanking sequence and that together the enzyme sets result in overlapping segments covering the entire insert. Therefore, at least one segment for each flank is of a predictable size and overlaps with another predictable size segment. This overlapping strategy confirms that the entire insert sequence is identified in a predictable hybridization pattern.

The results of these analyses of MON 87705 show that a single copy of the T-DNA is inserted at a single locus of the genome. Generational stability analysis demonstrated that an expected Southern blot fingerprint of MON 87705 has been maintained through four generations of the breeding history, thereby confirming the stability of the insert. Results from segregation analyses show heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

For each digest used to confirm copy number 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 bands present in the short run lanes, the molecular weight markers on the right of the figure were used.

The DNA sequencing analyses complement the Southern analyses. Southern analyses determined that MON 87705 contains T-DNA I and T-DNA II-derived sequences at a single insertion site. Sequencing of the insert and the flanking genomic DNA confirmed the organization of the elements within the insert, determined the 5' and 3' insert-to-plant junctions, determined the complete DNA sequence of the insert and adjacent soybean genomic DNA, and confirmed that the genomic DNA sequences flanking the 5' and 3' ends of the insert in MON 87705 are native to the soybean genome. Each genetic element is intact and the sequence of the insert matches the corresponding sequence in PV-GMPQ/HT4404. 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 (R3-R6) was also demonstrated by Southern blot fingerprint analyses. Four generations of MON 87705 were digested with one of the enzyme sets used for the copy number analysis and were hybridized with probes that would detect restriction segments that encompass the entire insert (two hybridization bands). This fingerprint strategy consists of two border segments that assess not only the stability of the insert, but also the stability of genomic DNA directly adjacent to the insert.

The Southern blot analysis confirmed the insert reported in Figure V-1 represents the only detectable insert in MON 87705. The genetic elements integrated in MON 87705 are summarized in Table V-2. Maps of plasmid vector PV-GMPQ/HT4404, used in the transformation to produce MON 87705 and annotated with the probes used in the Southern analysis are presented in Figures IV-1 and IV-2. Figure V-1 shows a linear map depicting restriction sites within the insert as well as within the known soybean genomic DNA immediately flanking the insert in MON 87705. Based on the linear map of the insert and the plasmid map, a table summarizing the expected DNA segments for Southern analyses is presented in Table V-1. In some of the Southern blots, the migration of the genomic DNA is slightly different when compared to the migration of the molecular weight markers. These altered migrations are likely the result of different base pair composition and/or differences in salt concentration between the genomic DNA samples and the molecular weight marker (Sambrook, 1989). The generations used in these studies are depicted in the breeding history shown in Figure V-7. Materials and methods used for characterization of the insert for MON 87705 are found in Appendix B.

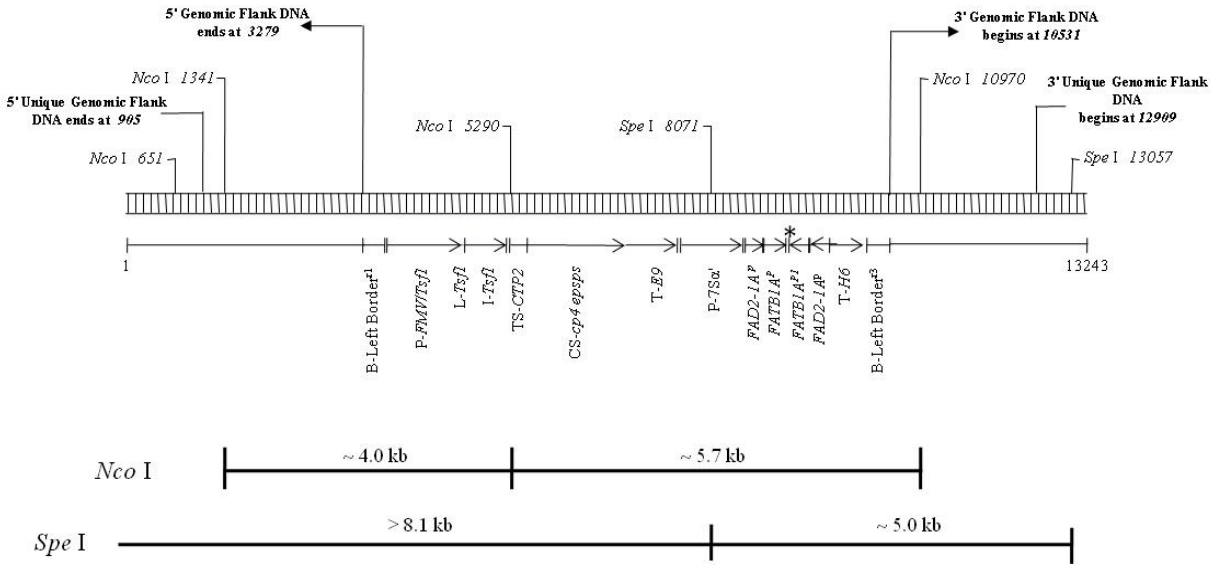


Figure V-1. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 87705

A linear map of the insert and genomic DNA flanking the insert in MON 87705 is shown. 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. Shown on the lower portion of the map are the expected sizes of the DNA segments after digestion with respective restriction enzymes. Arrowheads (→) indicate the end of the insert and the beginning of soybean genomic flanking sequence. The arrows (→) indicated the sequence direction of the elements in MON 87705. The * indicates partial sequences from the Left Border and Right Border sequences after integration into MON 87705 (Table V-2). Base pairs 906-3279 in the 5' flanking genomic DNA, and 10535-12908 in the 3' flanking genomic DNA represent duplicated bases from the 3′ end of the flanking soybean genomic DNA (see Section V.C).

Table V-1. Summary Chart of the Expected DNA Segments Based on Hybridizing Probes and Restriction Enzymes Used in MON 87705 Analysis

Southern blot Figure	**V-2**	**V-3**	**V-4**	**V-5**	**V-6**	**V-8**
Probes Used in Analysis	1, 4, 6	2, 5	3	7, 9	8, 10	1, 6
Positive Hybridization Controls						
Xho I + *Nco* I Digested Plasmid	~3.2, ~9.9 kb	~3.2, ~9.9 kb	~3.2 kb	~9.9 kb	~9.9 kb	~9.9 kb
Probe Templates [1]	~1.8, ~2.1, and ~1.1 kb	~0.8 and ~1.0 kb	~~[2]	~1.3 and ~1.5 kb	~1.9 and ~0.6 kb	~1.8 and ~1.1 kb
MON 87705 DNA Digestion						
Nco I	~4.0 and ~5.7 kb	~4.0 and ~5.7 kb	~5.7 kb	No band	No band	~4.0 and ~5.7 kb
Spe I	> 8.1* and ~5.0kb	> 8.1* and ~5.0kb	> 8.1* kb	No band	No band	--[3]

[1] probe templates were added to predigested conventional soybean DNA when multiple probes are used in Southern blot analysis.
[2] '~~' indicates that the plasmid template was the only positive control used, because the Southern blot was hybridized with one probe.
[3] '--' indicates that the particular restriction enzyme was not used in the analysis.
*Southern analysis indicates this segment to be ~ 11 kb.

Table V-2. Summary of Genetic Elements in MON 87705

Genetic Element[1]	Location in Sequence[2]	Function (Reference)
Unique 5' flanking sequence of the insert	1 – 905	Soybean genomic DNA
Sequence flanking 5' end of the insert	906-3279	2374 bp of soybean genomic DNA duplicated from the 3' end of the flanking sequence of the insert
B[3]-Left Border	3280 – 3538	259 bp sequence from the B-Left Border region remaining after integration (Barker et al., 1983)
Intervening Sequence	3539 – 3574	Sequence used in DNA cloning
P[4]-*FMV/Tsf1*	3575 – 4614	Chimeric promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic virus 35S RNA (Richins et al., 1987) combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
L[5]-*Tsf1*	4615 – 4660	5' untranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
I[6]-*Tsf1*	4661 – 5282	Intron with flanking exon sequence from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
Intervening Sequence	5283 – 5291	Sequence used in DNA cloning
TS[7]-*CTP2*	5292 – 5519	Targeting sequence from the *ShkG* gene encoding the transit peptide region of *Arabidopsis thaliana* EPSPS (Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS[8]-*cp4 epsps*	5520 – 6887	Codon modified coding sequence of the *aroA* gene from the *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgette et al., 1996c)
Intervening Sequence	6888 – 6945	Sequence used in DNA cloning

[3]**B –** Border; **P**[4] **–** Promoter; **L**[5] **–** Leader; **I**[6] **–** Intron; **TS**[7] **–** Targeting Sequence; **CS**[8] **–** Coding Sequence;

Table V-2 (cont.) Summary of Genetic Elements in MON 87705

Genetic Element	Location in Sequence	Function (Reference)
T^9-*E9*	6946 – 7588	3' untranslated region of the pea *RbcS2* gene which functions to direct polyadenylation of the mRNA (Coruzzi et al., 1984)
Intervening Sequence	7589 – 7676	Sequence used in DNA cloning
P-*7Sα'*	7677 – 8517	Promoter and leader from the *Sphas1* gene of *Glycine max* encoding beta-conglycinin storage protein (alpha'-bcsp) (Doyle et al., 1986) that directs transcription in seed
Intervening Sequence	8518 – 8539	Sequence used in DNA cloning
FAD2-1A^p	8540 – 8805	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
FATB1-A^p	8806 - 9106	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	9107 - 9114	Sequence used in DNA cloning
B-Right Border	9115 – 9134	20 bp sequence from the B-Right Border region remaining after integration (Zambryski et al., 1982)
B-Left Border	9135 - 9172	38 bp sequence from the B-Left Border region remaining after integration (Barker et al., 1983)
FATB1-A^{p1}	9173 – 9443	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
FAD2-1A^p	9444 – 9709	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	9710 – 9721	Sequence used in DNA cloning

T^9 – 3' untranslated transcriptional termination sequence and polyadenylation signal sequences; ***P*** – Partial sequence; ***P1*** – Truncated partial sequence of *FATB1-A*.

Table V-2 (cont.) Summary of Genetic Elements in MON 87705

Genetic Element	Location in Sequence	Function (Reference)
T-*H6*	9722 – 10157	3' UTR sequence of the *H6* gene from *Gossypium barbadense* encoding a fiber protein involved in secondary cell wall assembly (John and Keller, 1995)
Intervening Sequence	10158 –10255	Sequence used in DNA cloning
B-Left Border	10256 - 10530	275 bp sequence from the B-Left Border region remaining after integration (Adang et al., 1985)
Sequence flanking 3' end of the insert	10531 – 12908	Soybean genomic DNA including the 2374 bases duplicated at the 5' end of the flanking sequence of the insert
Unique 3' flanking sequence of the insert	12909 – 13243	Soybean genomic DNA

V.A. Copy Number of T-DNA I and T-DNA II in MON 87705

The copy number and insertion sites of T-DNA I and T-DNA II were assessed by digesting test DNA with restriction enzymes *Nco* I or *Spe* I and hybridizing Southern blots with probes that span T-DNA I and T-DNA II (Figures IV-1 and IV-2). Each restriction digest is expected to produce a specific banding pattern on the Southern blots (Table V-1). Since each detected segment contains flanking genomic DNA, any additional integrated sites would produce a different banding pattern with additional bands.

The restriction enzyme *Nco* I cuts once in the MON 87705 insert and once in each of the known 5' and 3' flanking sequences of MON 87705. Therefore, if T-DNA I and T-DNA II sequences are present at a single integration site in MON 87705, the digestion with *Nco* I was expected to generate two border segments with expected sizes of ~4.0 kb and ~5.7 kb (Figure V-1). The ~4.0 kb restriction segment contains genomic DNA flanking the 5' end of the insert, the Left Border, the *FMV/Tsf1* promoter, the *Tsf1* leader, and the *Tsf1* intron. The ~5.7 kb restriction segment contains the *CTP2* targeting sequence, *cp4 epsps* coding sequence, *E9* 3' untranslated sequence, *7Sα'* promoter, *FAD2-1A* and *FATB1-A* sense sequences, partial sequences of Right Border and Left Border, *FATB1-A* and *FAD2-1A* antisense sequences, *H6* 3' untranslated sequence, Left Border and genomic DNA flanking the 3' end of the insert.

The restriction enzyme *Spe* I cuts once in the MON 87705 insert and once in the known 3' flanking sequence of MON 87705. Therefore, if T-DNA I and T-DNA II sequences are present at a single integration site in MON 87705 digestion with *Spe* I is expected to release two border segments with expected sizes of ~5.0 kb and greater than 8.1 kb (Figure V-1). Since the *Spe* I site in the soybean genome flanking the 5' end of the insert lies outside of the known sequence, it was not possible to predict a precise segment size.

However, the segment size was determined by Southern blot analyses to be ~11 kb (Figures V-2, V-3, and V-4). The ~11 kb DNA segment contains genomic DNA flanking the 5' end of the insert, Left Border, *FMV/Tsf1* promoter, *Tsf1* leader, *Tsf1* intron, *CTP2* targeting sequence, *cp4 epsps* coding sequence, *E9* 3' untranslated sequence, and a portion of *7Sα'* promoter. The ~5.0 kb restriction segment contains the remaining portion of the *7Sα'* promoter, *FAD2-1A* and *FATB1-A* sense sequences, partial sequences of Right Border and Left Border, *FATB1-A* and *FAD2-1A* antisense sequences, *H6* 3' untranslated sequence, Left Border and genomic DNA flanking the 3' end of the insert.

In the Southern blot analyses performed, each Southern blot contained a negative and several positive controls. Conventional soybean DNA digested with *Nco* I or *Spe* I was used as a negative control to determine if the probes hybridized to any endogenous soybean sequences. As a positive control on the Southern blots, digested plasmid and probe templates were used. Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I was mixed with predigested conventional soybean genomic DNA and loaded on the gel. For Southern blots hybridized with multiple probes, each probe template was mixed with predigested conventional soybean DNA. The positive hybridization control was spiked at 0.1 and 1 genome equivalent to demonstrate sufficient sensitivity of the Southern blot. Individual Southern blots were hybridized with the following probe sets: Probes 1, 4, and 6; Probes 2 and 5; Probe 3; Probes 7 and 9; and Probes 8 and 10 (refer to Figures IV-1 and 2 and Table V-1). The results of this analysis are shown in Figures V-2 through V-6.

V.A.1. Probes 1, 4 and 6

Conventional soybean DNA digested with *Nco I* (Figure V-2, lanes 1 and 8) or *Spe* I (Figure V-2, lanes 3 and 10) and hybridized with the probes 1, 4, and 6 (Figure IV-1) produced several hybridization signals. These hybridization signals result from the probes (Probes 1, 4, and 6, Figure IV-1) hybridizing to endogenous sequences residing in the soybean genome and are not specific to the inserted DNA. These results were expected, because several genetic elements covered by probes 1, 4 and 6 are native to the soybean genome. These signals, as expected, were produced in both test and conventional soybean lanes, and therefore the bands are considered to be endogenous background hybridization.

Probe template spikes (Probes 1, 4 and 6, Figure IV-1) generated from plasmid PV-GMPQ/HT4404 were mixed with the conventional soybean DNA predigested with *Spe* I and produced the expected bands at ~1.8, ~2.1, and ~1.1 kb, respectively, (Figure V-2, lanes 5-6) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure V-2, lane 10). Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Spe* I (Figure V-2, lane 7) produced the expected size bands of ~3.2 and ~9.9 kb (refer to Figure V-1) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure V-2, lane 10). These results indicate that the probes are hybridizing to their target sequences.

MON 87705 DNA digested with *Nco I* (Figure V-2, lanes 2 and 9) produced two unique bands of ~4.0 and ~5.7 kb in addition to the endogenous background hybridization

observed in the conventional soybean DNA (Figure V-2, lanes 1 and 8). The ~4.0 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1). The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1).

MON 87705 DNA digested with *Spe* I (Figure V-2, lanes 4 and 11) produced two unique bands of ~5.0 and ~11 kb, in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure V-2, lanes 3 and 10). The ~11 kb segment is consistent with the expected band being greater than 8.1 kb. This band in the short run appears slightly larger, at ~13 kb, than the corresponding band in the long run. This border segment contains the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1). The ~5.0 kb band (Figure V-2, lane 4) is consistent with the expected band of 5.0 kb; however, the migration of the segment is slightly higher at ~5.2 kb in the short run (Figure V-2, lane 11) as indicated by the molecular weight marker. The ~5.0 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1).

There were no additional bands detected using the probes 1, 4, and 6. Based on the results presented in Figure V-2, it was concluded that T-DNA sequences covered by probes 1, 4, and 6 reside at a single integration locus in MON 87705.

V.A.2. Probes 2 and 5

Conventional soybean DNA digested with *Nco* I (Figure V-3, lanes 1 and 8) or *Spe* I (Figure V-3, lanes 3 and 10) and hybridized with probes 2 and 5 (Figure IV-1) produced several hybridization signals. These hybridization signals result from the probes (Probes 2 and 5, Figure IV-1) hybridizing to endogenous sequences residing in the soybean genome and are not specific to the inserted DNA. These results were expected, because several genetic elements covered by probes 2 and 5 are native to the soybean genome. These signals, as expected, were produced in both test and conventional soybean lanes, and therefore the bands are considered to be endogenous background.

Probe template spikes (Probes 2 and 5, Figure IV-1) generated from plasmid PV-GMPQ/HT4404 mixed with the conventional soybean DNA predigested with *Spe* I produced the expected bands at ~0.8 and ~1.0 kb (Figure V-3, lanes 5-6) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure V-3, lane 10). Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Spe* I (Figure V-3, lane 7) produced the expected size bands of ~3.2 and ~9.9 kb (refer to Figure V-1) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure V-3, lane 10). These results indicate that the probes are hybridizing to their target sequences.

MON 87705 DNA digested with *Nco* I (Figure V-3, lanes 2 and 9) produced two unique bands of ~4.0 and ~5.7 kb (Figure V-3, lanes 1 and 8). The ~4.0 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along

with the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1). The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1).

MON 87705 DNA digested with *Spe* I (Figure V-3, lanes 4 and 11) produced two unique bands of ~5.0 and ~11 kb (Figure V-3, lanes 3 and 10). The ~11 kb segment is consistent with the expected band being greater than 8.1 kb and with the ~11 kb segment seen with probes 1, 4, and 6 (Figure V-2, lanes 4 and 11). The ~11 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1). The ~5.0 kb border segment contains the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1). There were no additional bands detected using probes 2 and 5. Based on the results presented in Figure V-3, it was concluded that sequence covered by probes 2 and 5 resides at a single integration locus in MON 87705.

V.A.3. Probe 3

Conventional soybean DNA digested with *Nco* I (Figure V-4, lanes 1 and 7) or *Spe* I (Figure V-4, lanes 3 and 9) and hybridized with probe 3 (Figure IV-1) showed no detectable hybridization bands, as expected for the negative control.

Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Spe* I (Figure V-4, lanes 5-6) produced the expected size band of ~3.2 kb (refer to Figure V-1). This hybridization indicates that the probe is hybridizing to its target sequence.

MON 87705 DNA digested with *Nco* I (Figure V-4, lanes 2 and 8) produced the expected band of ~5.7 kb. The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1).

MON 87705 DNA digested with *Spe* I (Figure V-4, lanes 4 and 10) produced the expected band of ~11 kb. The ~11 kb band is consistent with the expected band being greater than 8.1 kb and with the ~11 kb segment seen in Figures V-2 and 3 (lanes 4 and 11). The ~11 kb band represents the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1).

There were no additional bands detected using probe 3. Based on the results presented in Figure V-4, it was concluded that sequence covered by probe 3 resides at a single detectable integration locus in MON 87705.

Taken together, the data presented in Figures V-2, V-3, and V-4 indicate that a single copy of the T-DNA I and T-DNA II sequences integrated into the soybean genome at a single detectable site in MON 87705.

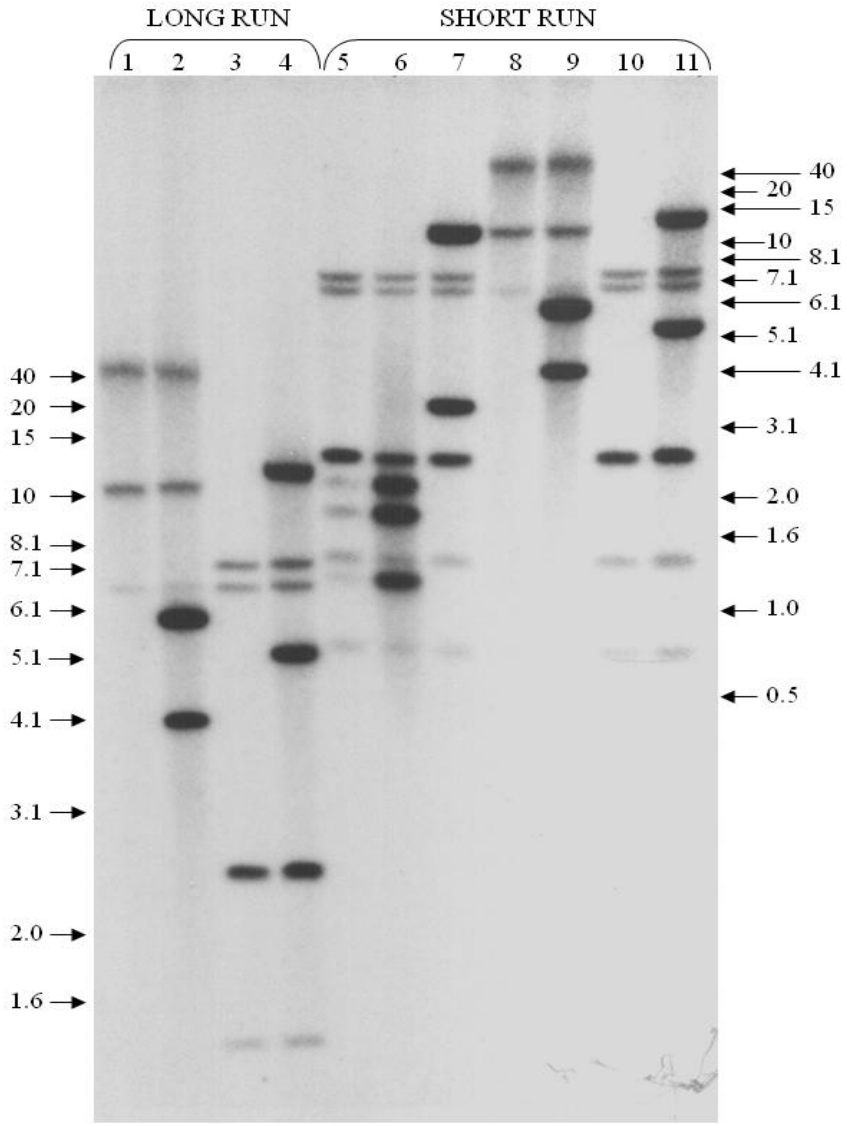


Figure V-2. Southern Blot Analysis of MON 87705: Probes 1, 4, and 6

The blot was hybridized with ^{32}P-labeled probes that span a portion of T-DNA I and T-DNA II sequences (probes 1, 4, and 6, Figure IV-1). Each lane contains ~10 μg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

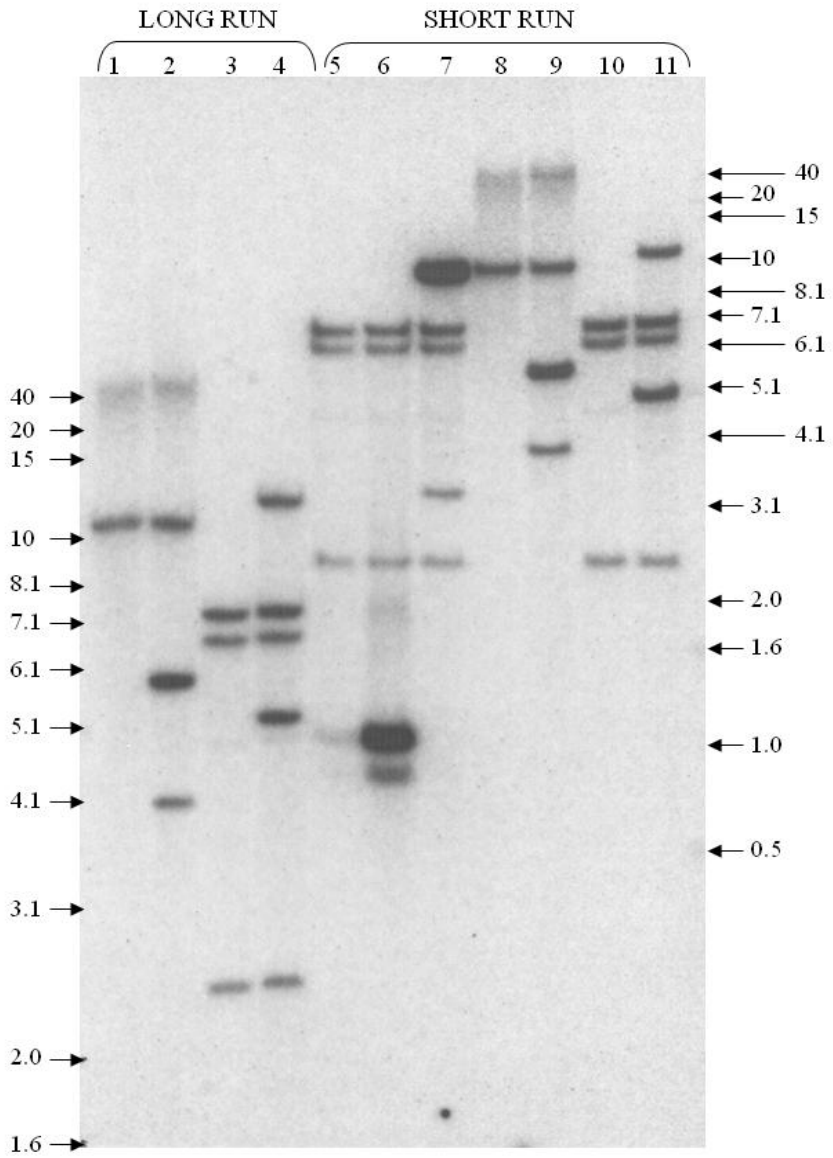


Figure V-3. Southern Blot Analysis of MON 87705: Probes 2 and 5

The blot was hybridized with ^{32}P-labeled probes that span a portion of T-DNA I sequences (probes 2 and 5, Figure IV-1). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

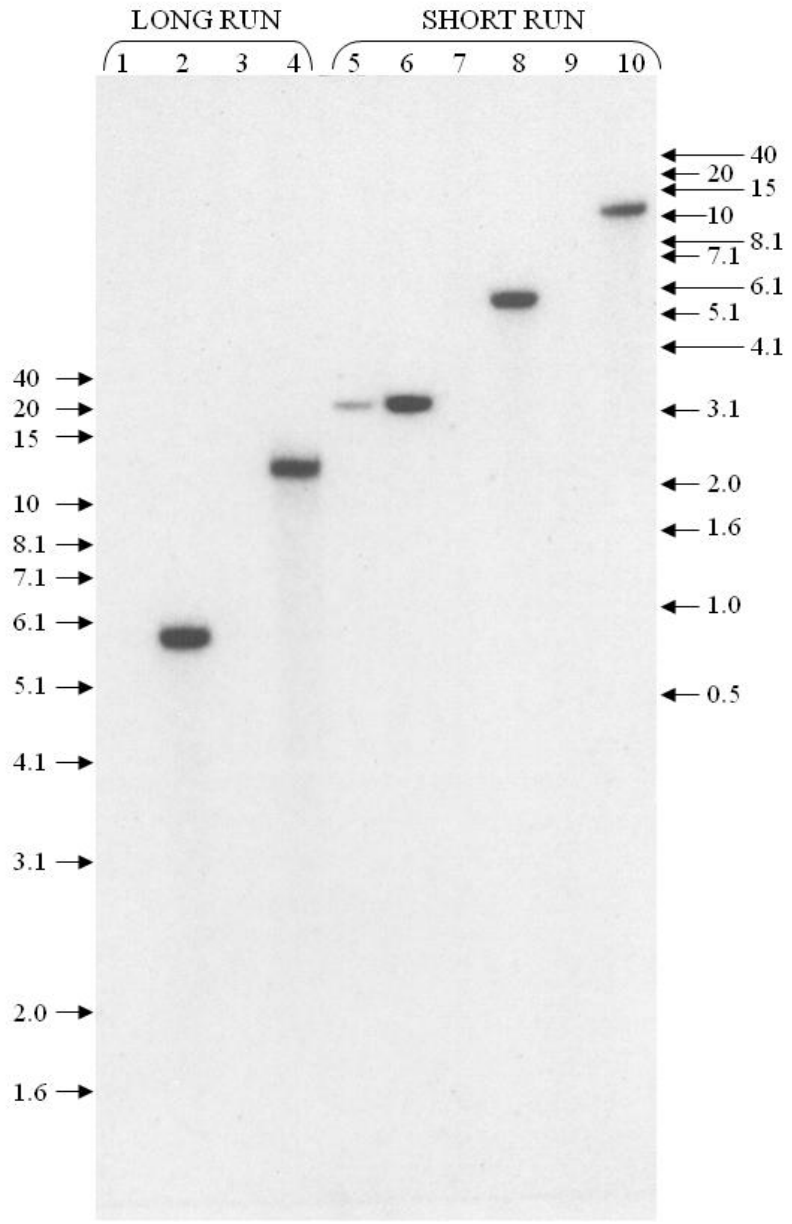


Figure V-4. Southern Blot Analysis of of MON 87705: Probe 3

The blot was hybridized with a ^{32}P-labeled probe that span a portion T-DNA I sequences (probe 3, Figure IV-1). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
7: Conventional soybean (*Nco* I)
8: MON 87705 (*Nco* I)
9: Conventional soybean (*Spe* I)
10: MON 87705 (*Spe* 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-GMPQ/HT4404 Backbone

MON 87705 and conventional soybean genomic DNA were digested with the restriction enzymes *Nco* I or *Spe* I. Probe template spikes (probes 7-10, Figure IV-2) generated from plasmid PV-GMPQ/HT4404 were mixed with the predigested conventional soybean genomic DNA to serve as positive hybridization controls. Additionally, plasmid PV-GMPQ/HT4404 DNA previously digested with the combination of *Xho* I and *Nco* I was mixed with conventional soybean DNA digested with *Spe* I and loaded on the gel to serve as a positive hybridization control. The blots were hybridized with probes 7-10 (Figure IV-2) that covered the entire backbone sequence of PV-GMPQ/HT4404. If backbone sequences are present in MON 87705, then probing with backbone sequence should result in unique hybridizing bands. The results are shown in Figures V-5 and V-6.

V.B.1. Plasmid Backbone Probes 7 and 9

Conventional soybean DNA digested with the restriction enzyme *Nco* I (Figure V-5, lanes 1 and 8) or *Spe* I (Figure V-5, lanes 3 and 10) and hybridized with probes 7 and 9 (Figure IV-2) showed no detectable hybridization bands, as expected for the negative control.

Probe template spikes (Probes 7 and 9, Figure IV-2) generated from plasmid PV-GMPQ/HT4404 and mixed with conventional soybean DNA predigested with *Spe* I produced the expected bands at ~1.3 and ~1.5 kb (Figure V-5, lanes 5 and 6). In addition, there is an unexpected faint band at ~3.0 kb (Figure V-5, lane 6). Based on size, this band is likely derived from dimers of the probe template (Qiagen at www.qiagen.com). Since this extra band only is present in the positive control and is not present in the conventional or MON 87705 DNA, it was concluded that the presence of this extra band did not impact or alter the final results for MON 87705. Plasmid PV-GMPQ/HT4404 digested with *Xho* I/*Nco* I and mixed with conventional soybean DNA digested with *Spe* I (Figure V-5, lane 7) produced an expected band that migrated at ~9.4 kb. This band is consistent with the expected band at ~9.9 kb; however, the migration of the ~9.4 kb segments is slightly lower than indicated by molecular weight marker most likely due to differences in salt concentrations between sample and marker. Overall, these results indicate that the probes are hybridizing to their target sequences.

MON 87705 DNA digested with either *Nco* I (Figure V-5, lanes 2 and 9) or *Spe* I (Figure V-5, lanes 4 and 11) showed no detectable hybridization signal, indicating that MON 87705 does not contain any detectable backbone sequence from the transformation vector PV-GMPQ/HT4404 that is covered by probes 7 and 9.

V.B.2. Plasmid Backbone Probes 8 and 10

Conventional soybean DNA digested with the restriction enzyme *Nco* I (Figure V-6, lanes 1 and 8) or *Spe* I (Figure V-6, lanes 3 and 10) and hybridized with probes 8 and 10

(Figure IV-2) showed no detectable hybridization bands, as expected for the negative control.

Probe template spikes (Probes 8 and 10, Figure IV-2) generated from plasmid PV-GMPQ/HT4404 and mixed with conventional soybean DNA predigested with *Spe* I produced two expected bands that migrated at ~0.7 and ~2.1 kb (Figure V-6, lanes 5 and 6). Plasmid PV-GMPQ/HT4404 digested with *Xho* I/*Nco* I and mixed with conventional soybean DNA digested with *Spe* I (Figure V-6, lane 7) produced an expected band that migrated at ~9.4 kb, which is consistent with the expected band at ~9.9 kb. The migration of the positive hybridization controls (Figure V-6, lanes 5-7) is slightly different than indicated by the molecular weight marker, most likely due to differences in salt concentrations between samples and markers. Overall, these results indicate that these probes are hybridizing to their control sequences.

MON 87705 DNA digested with either *Nco* I (Figure V-6, lanes 2 and 9) or *Spe* I (Figure V-6, lanes 4 and 11) showed no detectable hybridization signal. These results in combination with Figure V-5 indicate that MON 87705 does not contain any detectable backbone sequence from the transformation vector PV-GMPQ/HT4404.

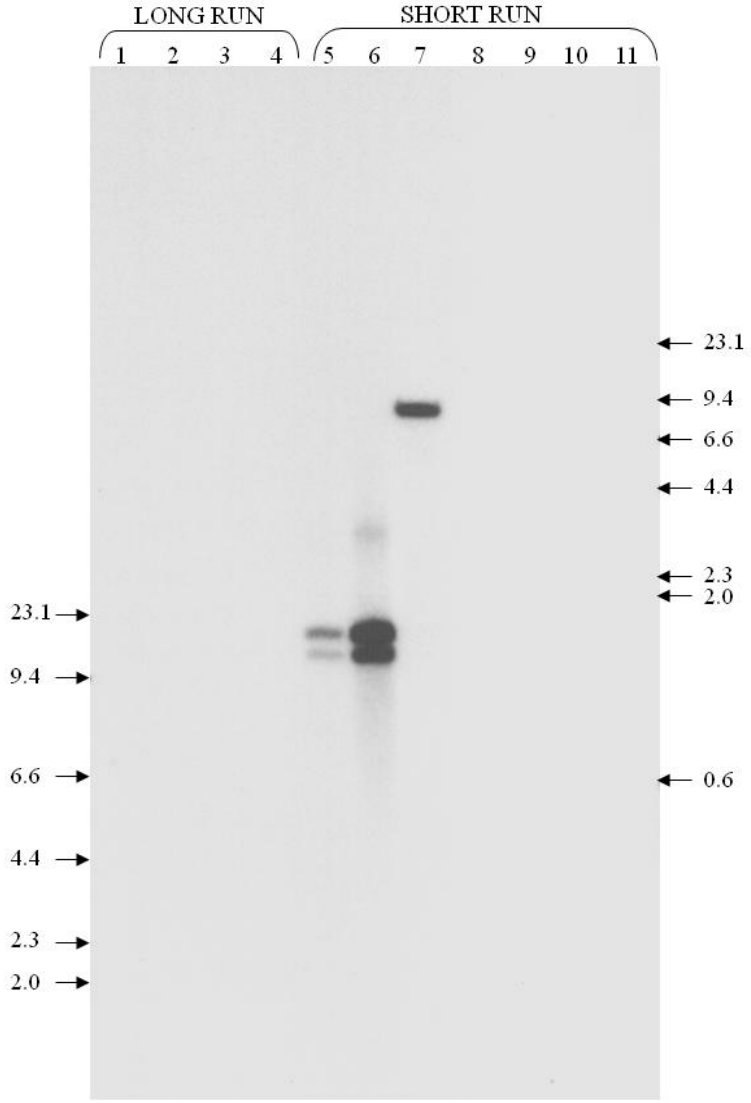


Figure V-5. Southern Blot Analysis of MON 87705:PV-GMPQ/HT4404 Backbone Probes 7 and 9

The blot was hybridized with ^{32}P-labeled probes that span a portion of backbone sequences (probes 7 and 9, Figure IV-2) of plasmid PV-GMPQ/HT4404. Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

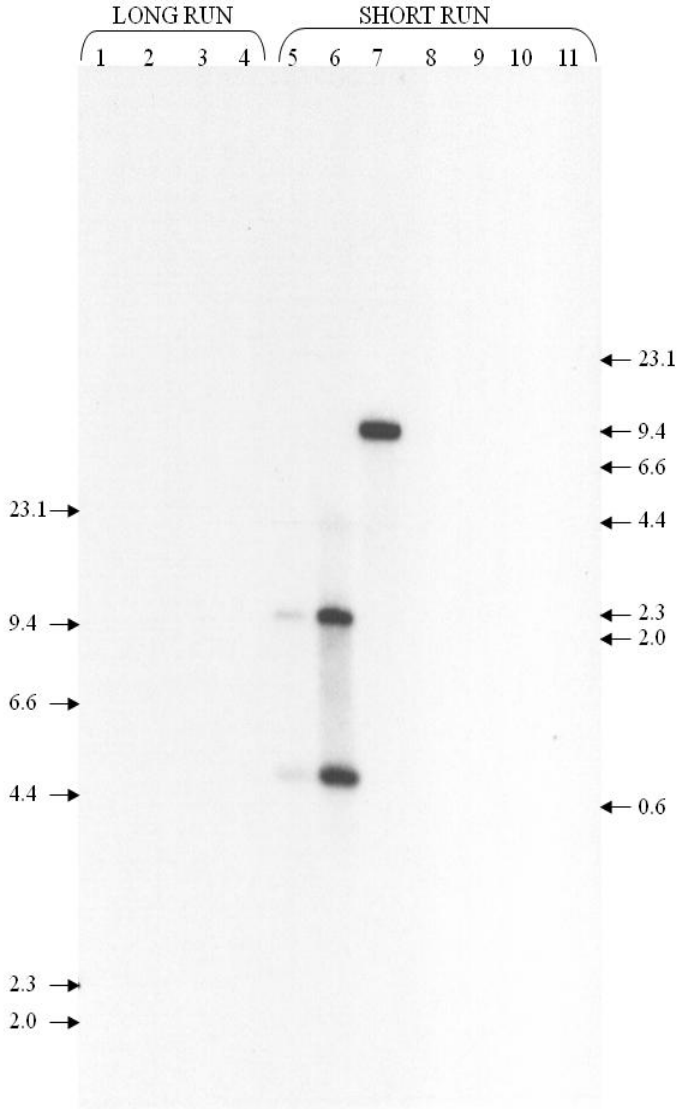


Figure V-6. Southern Blot Analysis of MON 87705: PV-GMPQ/HT4404 Backbone Probes 8 and 10

The blot was hybridized with ^{32}P-labeled probes that span a portion of backbone sequences (probes 8 and 10, Figure IV-2) of plasmid PV-GMPQ/HT4404. Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

V.C. Organization and Sequence of the Insert and Adjacent Genomic DNA in MON 87705

The organization of the elements within the MON 87705 insert was confirmed by DNA sequence analyses. Several PCR primers were designed with the intent to amplify six overlapping regions of DNA that span the entire length of the insert (see Appendix B). The amplified DNA segments were subjected to DNA sequencing analyses. The insert in MON 87705 is 7251 base pairs and matches the sequence of PV-GMPQ/HT4404 as described in Tables IV-3 and V-2.

A sequence comparison between the PCR product generated from the conventional soybean (A3525) and the sequence generated from the 5' and 3' flanking sequences of MON 87705 indicates there was a 36 bp deletion (bases 896-931) and a 2374 bp insertion just 5' to the MON 87705 insertion site. Given the very high homology between the 2374 bases flanking the 5' end of the insert and the genomic DNA flanking the 3' end of the insert, the 2374 bases are most likely from the 3' end of the flanking genomic DNA and were duplicated at the 5' end of the insertion site when T-DNA I and T-DNA II integrated into the genome. This duplication has the following characteristics: 1) there is a single nucleotide change detected in the duplicated 2374 base pairs at the 5' flanking sequence; and 2) there are four unique bases located at the junction of the insert DNA and the 2374 bases in the 3' flanking sequence. This analysis confirms that the genomic sequences flanking the insert in MON 87705 are native to the soybean genome and that a 36 base-pair deletion and a 2374 base pair duplication that contains a single base change occurred at the insertion site during integration of the T-DNA sequences. These molecular rearrangements presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998).

V.D. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 87705

In order to demonstrate the stability of the T-DNA I and T-DNA II insert in MON 87705 in multiple generations, Southern blot analyses were performed using DNA obtained from multiple generations of the MON 87705 breeding history. For reference, the breeding history of MON 87705 is presented in Figure V-7. The specific generations tested are indicated in the legends of Figure V-8. DNA samples from R3, R4, R5, and R6 generations of MON 87705 (refer to Figure V-7) were digested with *Nco* I and were expected to release two border segments with the expected sizes of 4.0 and 5.7 kb (Figure V-1). The detected hybridization bands in R4, R5, and R6 generations are compared to the fully characterized MON 87705 R3 generation to evaluate stability. Any instability associated with the insert would be detected as faint novel bands within the fingerprint on the Southern blot. The blot was hybridized simultaneously with two radiolabeled probes that cover both border segments generated by the digest (probes 1 and 6, Figure IV-1). This blot has two of the same positive hybridization controls (probes 1 and 6, Figure IV-1) as described in Section V.A.1. The result of this analysis is shown in Figure V-8.

Conventional soybean DNA digested with *Nco* I and hybridized with probes 1 and 6 (Figure V-8, lane 4) showed hybridization bands. These hybridization signals result from the probes hybridizing to endogenous targets residing in the soybean genome and are not specific to the inserted DNA.

Probe templates spikes (Probes 1 and 6, Figure IV-1) generated from plasmid PV-GMPQ/HT4404 and mixed with conventional soybean DNA predigested with *Nco* I (Figure V-8, lanes 1 and 2) produced the expected size bands at ~1.8 and ~1.1 kb. The detection of the probe template positive hybridization controls demonstrates that both probes were hybridizing to the target DNA. Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Nco* I (Figure V-8, lane 3) produced the expected size band at ~9.9 kb, which indicates that the probes are hybridizing to their corresponding sequence in the transformation vector. This expected band at ~9.9 kb migrated together with an endogenous hybridization signal observed in Figure V-8, lane 4.

Digestion of MON 87705 from multiple generations (refer to Breeding History of MON 87705, Figure V-7) with restriction enzyme *Nco* I produced two bands at ~4.0 and ~5.7 kb (Figure V-8, lanes 5-8) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure V-8, lane 4). The ~4.0 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1). The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1). However, the migration of this segment appears slightly lower than indicated by the molecular weight marker most likely due to differences in salt concentrations between the samples and marker. This restriction pattern is the same as the restriction pattern observed in the Southern blot analysis of the R3 generation shown in Figure V-2 (lanes 2 and 9).

There were no additional unexpected bands detected, indicating that the single copy of T-DNA I and T-DNA II in MON 87705 is stably maintained in the selected generations.

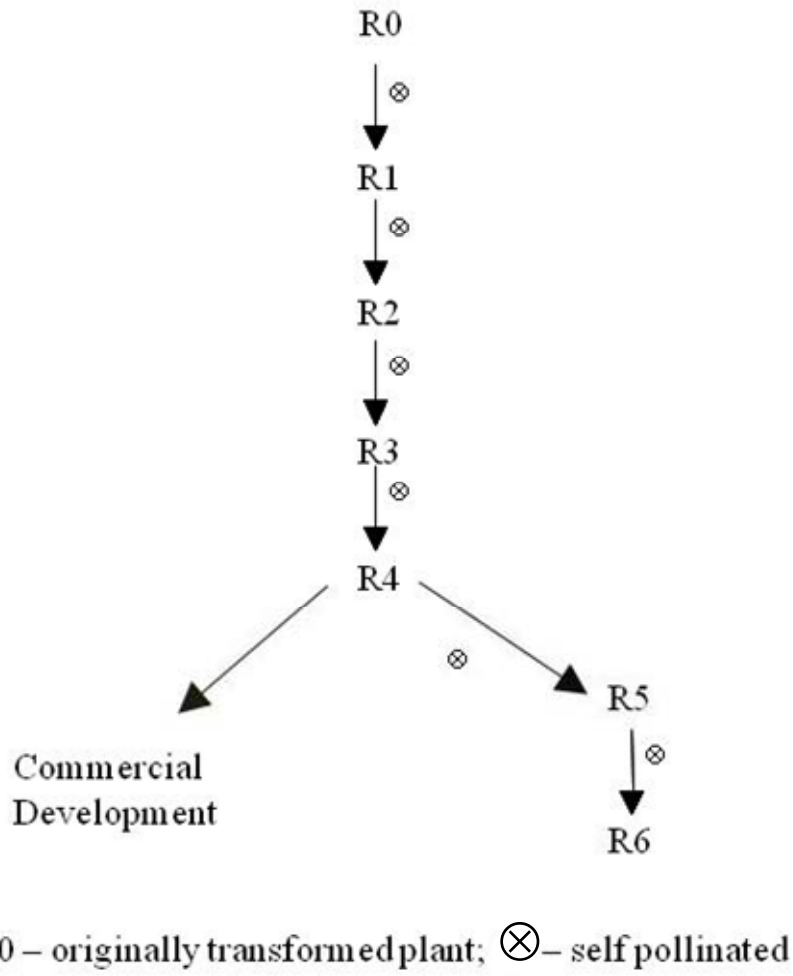


Figure V-7. Breeding History of MON 87705
All generations were self pollinated (⊗). The R3 generation was used for the molecular analyses of MON 87705 reported in Figures V-2 through V-6 and is referred to as MON 87705 in all Southern blot figures. The R3, R4, R5, and R6 generations were used for analyzing the stability of the insert in multiple generations.

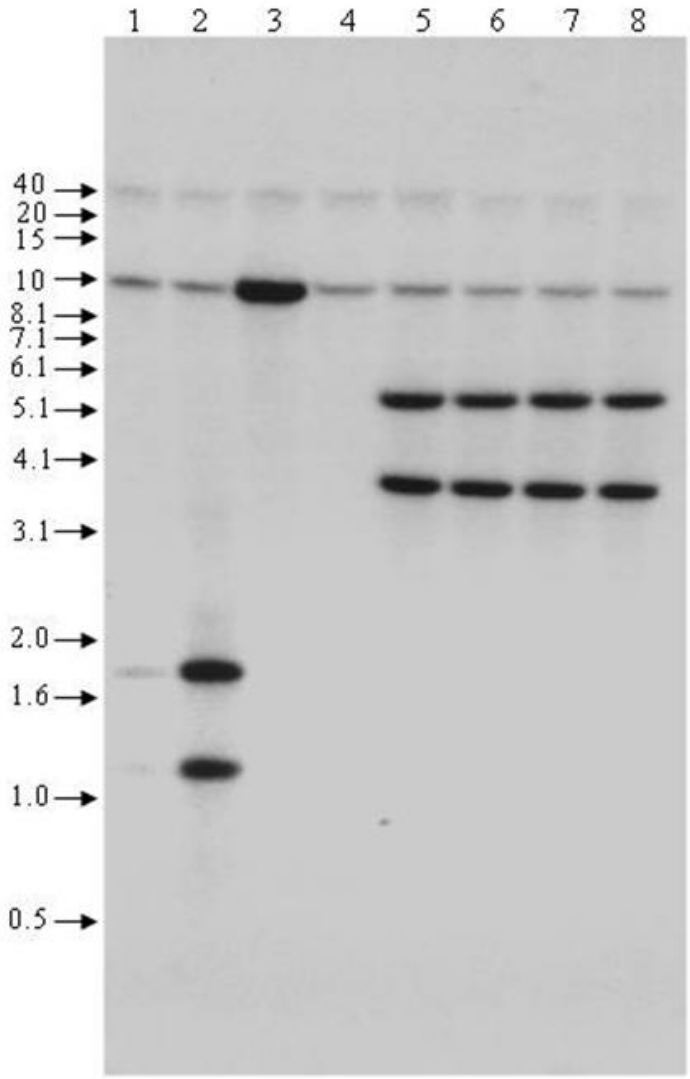


Figure V-8. Generational Stability of MON 87705: Probes 1 and 6

The blot was hybridized with ^{32}P-labeled probes that spanned a portion of T-DNA I and T-DNA II sequences (probes 1, and 6, Figure IV-1). Each lane contains ~ 10 µg of digested genomic DNA isolated from leaf tissue. The breeding history of MON 87705 is illustrated in Figure V-7. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I) spiked with probe templates [~0.1 genomic equivalent]
2: Conventional soybean (*Nco* I) spiked with probe templates [~1 genomic equivalent]
3: Conventional soybean (*Nco* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
4: Conventional soybean (*Nco* I)
5: MON 87705 [R3, (*Nco* I)]
6: MON 87705 [R4, (*Nco* I)]
7: MON 87705 [R5, (*Nco* I)]
8: MON 87705 [R6, (*Nco* I)]

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

V.E. Inheritance of the Genetic Insert in MON 87705

During development of MON 87705 segregation data were recorded to assess the heritability and stability of the coding sequences present in MON 87705. Chi-square analysis was performed over several generations to confirm the segregation and stability of the MON 87705 insertion. The Chi-square analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 87705 breeding path from which segregation data were generated is described in Figure V-9. The transformed R0 plant was self-pollinated to produce R1 seed. From the R1 segregating population, an individual plant (#90, designated MON 87705) homozygous for a single copy of the *H6* 3'UTR was identified via Invader (Wave Technologies, Inc.) and Southern blot analysis.

The selected R1 MON 87705 plant was self-pollinated to give rise to a population of R2 plants that were repeatedly self-pollinated through to the R4 generation. At each generation, the fixed homozygous plants were tested for the expected segregation pattern of 1:0 (positive: negative) for the *H6* 3'UTR using Invader analysis.

At the R4 generation, homozygous MON 87705 plants were bred via traditional breeding with a soybean variety that did not contain the *H6* 3'UTR to produce F1 hemizygous seed. The resulting F1 plants were then self-pollinated to produce F2 seed. The heritability and stability of the coding sequences present in MON 87705 were assessed from plants of the F2, F3, F4, and F5 generations. At each of these generations, the plants were tested for the presence of the *H6* 3'UTR by Invader analysis, and hemizygous positive plants were then selected and self-pollinated to produce seed of the next generation.

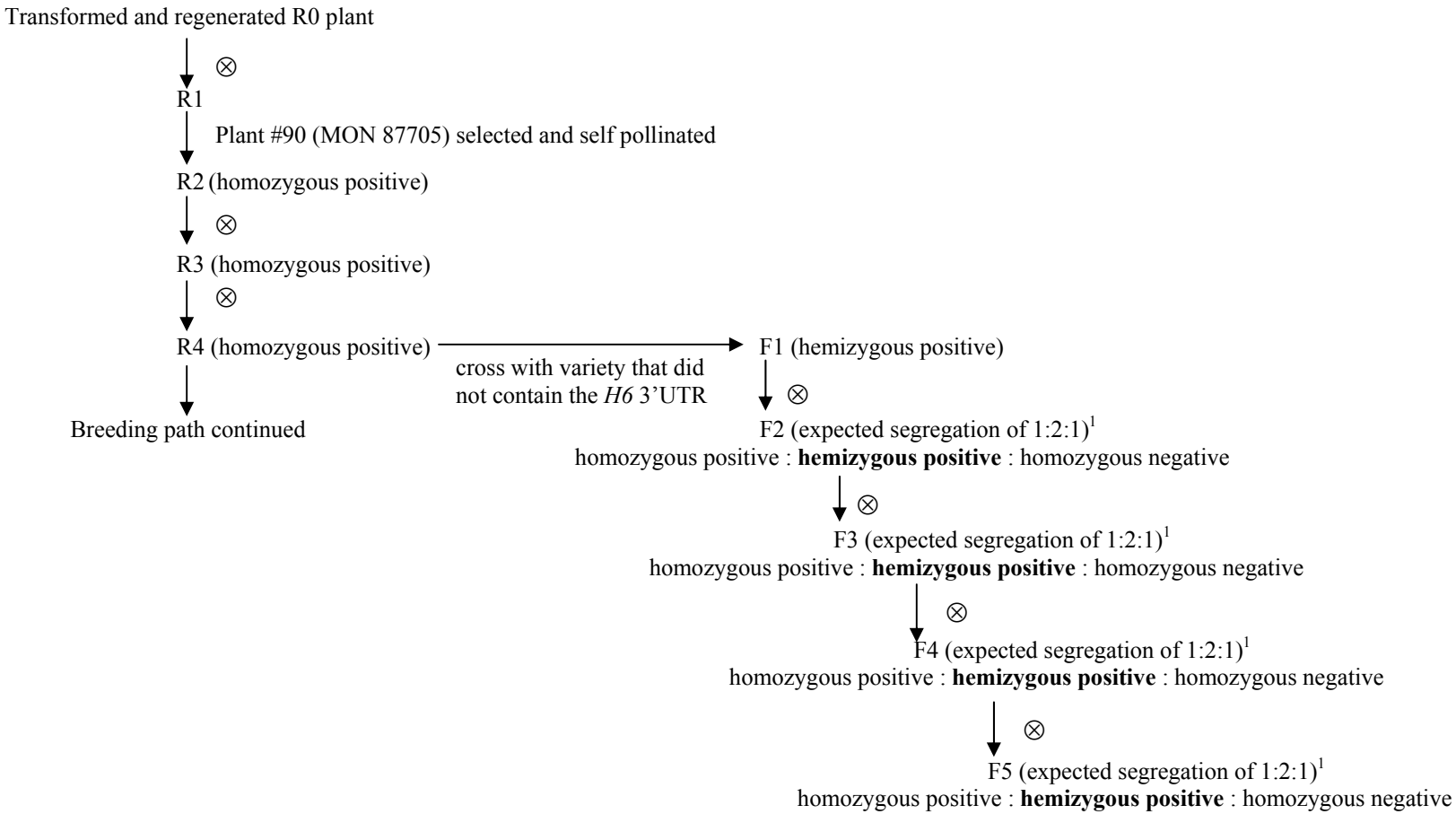
A Chi-square (χ^2) analysis was used to compare the observed segregation ratios to the expected ratios according to Mendelian principles. The Chi-square value was calculated as:

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

where o = observed frequency of the genotype and e = expected frequency of the genotype. 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 87705 are presented in Table V-3. The χ^2 values in the F2 generation indicated no statistically significant difference between the observed and expected 1:2:1 (homozygous positive:hemizygous positive:homozygous negative) segregation ratio. The χ^2 value in the F3 generation indicated a statistically significant difference between the observed and expected 1:2:1 segregation ratio. However, there were ten plants out of 91 total plants tested in the assay in which the zygosity could not be determined by Invader analysis. These missing data combined with a relatively small sample size (n=81) of tested plants may have skewed the segregation ratio. This caused the results of the analysis to be inconclusive and the data from the F3 generation could not be used to accurately assess the heritability and stability of the coding sequences present in MON 87705. Therefore, the F4 and F5

generations were tested using larger sample sizes to further assess the heritability and stability of the inserted coding sequences. The χ^2 values in the F4 and F5 generations indicated no statistically significant differences between the observed and expected 1:2:1 segregation ratios. Considering the data from three generations (F2, F4, and F5), the results support the conclusion that the coding sequences present in MON 87705 reside at a single locus within the soybean genome and are inherited according to Mendelian inheritance principles. These results are also consistent with the molecular characterization data that indicate a single genomic insertion site for the coding sequences present in MON 87705 that encode for the improved fatty acid profile and glyphosate tolerance trait (Table VI-1).



⊗ = Self pollinated

Figure V-9. Breeding Path for Generating Segregation Data MON 87705

[1] Chi-square analysis conducted on segregation data from the F2, F3, F4 and F5 generations

Table V-3. Segregation of the H6 3'UTR Gene During the Development of MON 87705

					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
F2	4197	1009	2091	1097	1049.25	2098.5	1049.25	3.7	0.1538
F3	81	30	35	16	20.25	40.5	20.25	6.3	0.0421
F4	266	68	126	72	66.5	133	66.5	0.9	0.6514
F5	175	44	88	43	43.75	87.5	43.75	0.0	0.9915

[1] F2 progeny were from the cross of MON 87705 homozygous positive for the *H6* 3'UTR with a soybean variety that did not contain the *H6* 3'UTR. F3, F4, and F5 progeny were from self-pollinated plants of the previous generation that were hemizygous positive for the *H6* 3'UTR.
[2] Plants were tested for the presence of the *H6* 3'UTR by Invader analysis. "Total plants" refers to the total number of plants in which zygosity could be determined using the assay.

V.F. Conclusion of Molecular Characterization

Molecular characterization of MON 87705 by Southern blot analyses demonstrated that a single copy of the T-DNA I and T- DNA II sequences from the transformation vector PV-GMPQ/HT4404 was integrated into the soybean genome at a single locus. There were no additional genetic elements, including backbone sequences, from the transformation vector PV-GMPQ/HT4404 detected, linked or unlinked to the intact DNA insert, in MON 87705.

PCR and DNA sequence analyses were performed on MON 87705, which confirmed the organization of the elements within the insert, determined the 5' and 3' insert-to-plant junctions, determined the complete DNA sequence of the insert and adjacent soybean genomic DNA sequence in MON 87705, and confirmed that the genomic DNA sequences flanking the 5' and 3' ends of the insert in MON 87705 are native to the soybean genome. The PCR and DNA sequence analysis identified 36 bp of conventional soybean DNA sequence deleted at the insertion site in MON 87705. Additionally, a 2374 bp duplication was identified in the 5' genomic flanking sequence of MON 87705. This duplicated sequence is likely from the 3′ flanking sequence of MON 87705 and it contains a single nucleotide change.

Generational stability analysis demonstrated that an expected Southern blot fingerprint of MON 87705 has been maintained through four generations of the breeding history, thereby confirming the stability of the insert. Results from segregation analyses show heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

VI. CHARACTERIZATION OF THE INTRODUCED CP4 EPSPS PROTEIN IN MON 87705

As described in Section V, the MON 87705 insert contains a *cp4 epsps* expression cassette and a *FATB/FAD2* supression cassette. As the *FATB/FAD2* cassette does not encode for any proteins, this section focuses on the characterization and safety of the CP4 EPSPS protein produced by MON 87705.

The RNA-based suppression of *FATB* and *FAD2* soybean genes in MON 87705 is mediated by dsRNA molecules. Double stranded RNAs are commonly found in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids. Nucleic acids have a long history of safe consumption and are considered GRAS by the U.S. Food and Drug Administration. There is no evidence to suggest dietary consumption of RNA is associated with toxicity or allergenicity. Moreover, analysis of the DNA segments encoding this dsRNA showed that they lack the sequences required for translation initiation and protein synthesis. The production of a protein from the dsRNA encoded by the insert in MON 87705 is highly unlikely. Several biotechnology-derived plant products previously deregulated by APHIS were developed using RNA-based suppression mechanisms, including virus-resistant papaya and squash, high oleic soybean, FLAVR SAVR tomatoes, and plum trees resistant to Plum pox virus. Based on this information, it is concluded that the inserted DNA and resulting dsRNA are safe and unlikely to produce a protein or polypeptide. As a result, the RNA-based suppression technology used in MON 87705 poses no novel risks from a food, feed or environmental perspective (FDA, 1994; USDA-APHIS, 1994; USDA-APHIS, 1997; USDA-APHIS, 2006; USDA-APHIS, 2007).

The remainder of this section summarizes the assessment of the CP4 EPSPS protein produced in MON 87705 including: 1) the identity of the CP4 EPSPS protein from MON 87705; 2) demonstration of the equivalence of the plant-produced and *E. coli*-produced CP4 EPSPS proteins used in laboratory and regulatory safety evaluations; 3) the CP4 EPSPS protein expression levels in MON 87705 soybean tissues; and 4) an allergenicity assessment for the CP4 EPSPS protein. Results indicate that the MON 87705-produced CP4 EPSPS 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 and Function of the CP4 EPSPS Protein from MON 87705

The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS: EC2.5.1.19) family of enzymes is ubiquitous to plants and microorganisms. EPSPS proteins have been isolated from both sources, and their properties have been extensively studied (Harrison et al., 1996; Haslam, 1993; Klee et al., 1987; Schonbrunn et al., 2001; Steinrucken and Amrhein, 1984). The shikimate pathway and the EPSPS protein are absent in mammals, fish, birds, reptiles, and insects (Alibhai and Stallings, 2001). The bacterial and plant enzymes are mono-functional with a molecular weight of 44-48 kDa (Kishore et al., 1988). EPSPS proteins catalyze the transfer of the enolpyruvyl group from phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (EPSP) (Alibhai

and Stallings, 2001). Due to the specificity of EPSPS for its substrates, the only known catalytic product generated is EPSP, which is the penultimate product of the shikimic acid pathway. Shikimic acid is a substrate for the biosynthesis of the aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules. It has been estimated that aromatic molecules, all of which are derived from shikimic acid, represent 35% or more of the dry weight of a plant (Franz et al., 1997).

The EPSPS transgene in MON 87705 is derived from *Agrobacterium* sp. strain CP4 (*cp4 epsps*). The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996b). The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in the Roundup family of agricultural herbicides, relative to endogenous plant EPSPS (Padgette et al., 1996b). In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of shikimate-3-phosphate, thereby depriving plants of essential amino acids (Haslam, 1993; Steinrücken and Amrhein, 1980). In Roundup Ready plants, which are tolerant to the Roundup family of agricultural herbicides, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996b). The CP4 EPSPS protein expressed in MON 87705 is identical to the CP4 EPSPS protein in other Roundup Ready crops including Roundup Ready soybean (404-3-2), Roundup Ready 2 Yield soybean (MON 89788), Roundup Ready Corn 2, Roundup Ready canola, Roundup Ready sugar beet, and Roundup Ready cotton.

VI.B. Characterization of the Full Length CP4 EPSPS Protein from MON 87705

The safety assessment of crops derived through biotechnology includes characterization of the introduced protein produced from the inserted DNA, confirmation of its functional and physicochemical properties, and confirmation of the safety of the protein. The level of CP4 EPSPS protein produced in MON 87705 is too low to allow purification of sufficient quantities for use in subsequent safety assessment studies. Therefore, it is necessary to produce the protein in high-expressing recombinant host systems (such as bacteria) in order to obtain sufficient quantities of the CP4 EPSPS protein. CP4 EPSPS protein was produced in *E. coli*, and subsequently purified and characterized. A small quantity of the CP4 EPSPS protein was also purified from harvested MON 87705 seed. The equivalence of the physicochemical characteristics and functional activity between the MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins was confirmed by a panel of analytical techniques, including: (1) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish equivalence of the apparent molecular weight between MON 87705-produced and the *E. coli*-produced reference standard protein, (2) western blot analysis to establish immunoreactive equivalence between MON 87705-produced and the *E. coli*-produced reference standard protein using an anti-CP4 EPSPS antibody, (3) N-terminal sequence analysis, (4) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to generate a tryptic peptide map of MON 87705-produced CP4 EPSPS, (5) CP4 EPSPS enzymatic activity analysis to demonstrate functional equivalence between MON 87705-produced and the *E. coli*-produced reference standard protein, and (6) glycosylation analysis to

establish equivalent glycosylation status between MON 87705-produced and *E. coli*-produced reference standard protein. The details of the materials, methods, and results are described in Appendix C while the conclusions are summarized below.

A comparison of the MON 87705-produced CP4 EPSPS to the *E. coli*-produced CP4 EPSPS reference standard protein confirmed the identity of the MON 87705-produced CP4 EPSPS protein and established the equivalence of the plant produced protein to the *E. coli*-produced CP4 EPSPS reference standard protein. The molecular weight of the MON 87705-produced and *E. coli*-produced CP4 EPSPS protein was estimated by SDS-PAGE. The SDS-PAGE demonstrated that the proteins migrated identically, indicating that the CP4 EPSPS proteins from both sources are equivalent in their molecular weight. The electrophoretic mobility and immunoreactive properties of the MON 87705-produced CP4 EPSPS protein were shown to be equivalent to those of the *E. coli*-produced CP4 EPSPS protein reference standard by immunoblot. The N-terminus of the MON 87705 produced CP4 EPSPS protein was consistent with the predicted amino acid sequence translated from the CP4 EPSPS coding sequence, and the MALDI-TOF MS analysis yielded peptide masses consistent with the expected peptide masses from the translated CP4 EPSPS coding sequence. The MON 87705- produced and the *E. coli*-produced CP4 EPSPS protein reference standard were also found to be equivalent based on the functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CP4 EPSPS protein isolated from MON 87705 and established its equivalence to the *E. coli*-produced CP4 EPSPS reference standard protein. Furthermore, since CP4 EPSPS proteins isolated from other Roundup Ready crops (Roundup Ready soybean, Roundup Ready 2 Yield soybean, Roundup Ready canola, Roundup Ready cotton and Roundup Ready sugar beet) have established equivalence to the *E. coli*-produced protein standard, by inference, the MON 87705-derived CP4 EPSPS protein is likely to possess equivalent biochemical and physiological characteristics with the CP4 EPSPS proteins expressed in other Roundup Ready crops, all of which have been deregulated by USDA-APHIS.

VI.C. Expression Levels of CP4 EPSPS Protein in MON 87705

CP4 EPSPS protein levels in various tissues of MON 87705 that are relevant to the risk assessment were assessed by a validated enzyme-linked immunosorbent assay (ELISA). Tissues of MON 87705 and conventional control were collected during the 2007/2008 growing season from five field sites in Chile (City, Province): Quilapilum, Chacabuco; Melipilla, Melipilla; Calera de Tango, Maipo; Rancagua, Cachapoal; and San Fernando, Colchagua. These field sites were representative of soybean producing regions suitable for commercial production. At each site, three replicated plots of plants containing MON 87705, as well as a conventional soybean control, were planted using a randomized complete block field design. Over-season leaf (OSL 1-4), root, forage, and mature seed tissues were collected from each replicated plot at all field sites. A description of tissues collected is provided below.

Table VI-1. Tissues collected for MON 87705

Tissue	Soybean development stage	Days after planting (DAP)
OSL-1	V2-V3	31-35
OSL-2	V7	46-50
OSL-3	V10	62-66
OSL-4	V14	84-88
Forage	R5-R6	101-106
Root	R5-R6	101-106
Mature Seed	R8	154-158

The CP4 EPSPS protein levels were determined in all seven tissue types described above. The results obtained from ELISA analysis are summarized in Table VI-2 and the details of the materials and methods are described in Appendix D. In summary, the 2007/2008 Chilé expression study showed the CP4 EPSPS protein in MON 87705 was detected in all tissue types across all five sites with a range from 40 – 1000 µg/g dwt. The levels of the CP4 EPSPS protein from the conventional control (A3525) were less than the assay limits of detection (LOD) or limit of quantitation LOQ in all tissue types. The mean CP4 EPSPS protein levels across the five sites were highest in leaf (ranging from OSL-1 200 µg/g dwt to OSL-2 530 µg/g dwt), followed by root (120 µg/g dwt), seed (110 µg/g dwt) and forage (77 µg/g dwt).

Table VI-2. Summary of CP4 EPSPS Protein Levels in Leaf, Seed, Root, and Forage Tissues from MON 87705 Grown in 2007/2008 Chile Field Trials

Tissue Type[1]	CP4 EPSPS protein µg/g fwt[2] (SD)[3]	Range[4] µg/g fwt[2]	CP4 EPSPS protein µg/g dwt[5] (SD)[2]	Range[4] µg/g dwt[5]	LOQ/LOD µg/g fwt[2]
OSL-1	36 (14)	16-65	200 (72)	84-340	0.57/0.26
OSL-2	110 (51)	60-230	530 (230)	290-1000	0.57/0.26
OSL-3	51 (21)	11-84	220 (94)	47-350	0.57/0.26
OSL-4	51 (21)	27-94	210 (92)	110-410	0.57/0.26
Root	32 (5.3)	22-40	120 (24)	77-160	0.57/0.10
Forage	24 (6.4)	14-34	77 (24)	41-120	0.57/0.11
Mature Seed	100 (39)	35-190	110 (44)	40-210	0.34/0.26

[1]The OSL-1, OSL-2, OSL-3, OSL-4 samples were collected approximately at V2 – V3, V7, V10; and V14 stages, respectively. The forage and root were collected approximately at R5-R6 stage, and the mature seed was collected at R8 stage.

[2]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.

[3]The means and standard deviations were calculated for each tissue type across all sites (n=15 for all tissues, except OSL-2 where n=12 and OSL-3 where n=19).

[4]Minimum and maximum values were determined for each tissue type across all sites.

[5]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a dry weight (dwt) basis. The dry weight values were calculated by dividing the µg/g fwt by the dry weight conversion factors obtained from moisture analysis data.

VI.D. Assessment of Potential Allergenicity of the CP4 EPSPS Protein from MON 87705

According to guidelines adopted by the Codex Alimentarius Commission (Codex, 2003) for the allergy safety evaluation 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, 2003). A protein is not likely to be associated with allergenicity if: 1) the protein is from a nonallergenic 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 CP4 EPSPS protein in MON 87705 has been assessed for its potential allergenicity according to these safety assessment guidelines.

The CP4 EPSPS protein is from *Agrobacterium* sp., strain CP4, an organism that is not a source of known allergens. Bioinformatics analyses demonstrated that the CP4 EPSPS protein does not share 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 CP4 EPSPS protein demonstrated that the full-length protein is rapidly digested in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF) *in vitro* assays (Harrison et al., 1996). Finally, the CP4 EPSPS protein represents no more than 0.031% of the total protein in the seed of MON 87705, a relatively low abundance for this protein compared to the rest of the seed protein content. Taken together, these data support the conclusion that the CP4 EPSPS protein present in MON 87705 is not similar to known allergens and does not pose a significant allergenic risk to humans or animals.

VI.E. Safety Assessment Summary of CP4 EPSPS Protein

The U.S. Environmental Protection Agency (EPA) has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (EPA, 1996). This 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. Since the MON 87705-produced CP4 EPSPS protein is equivalent to the exempted CP4 EPSPS protein, a similar conclusion can be reached that the MON 87705-produced CP4 EPSPS is safe for human and animal consumption. The comprehensive food and feed safety and nutritional assessment of MON 87705 also is scheduled to be submitted to the FDA, which will include the following conclusions:

a) The donor organism, *Agrobacterium* species strain CP4 is not known for human or animal pathogenicity, and is not commonly allergenic. *Agrobacterium* sp. strain CP4 has been previously reviewed as a part of the safety assessment of the donor organism during Monsanto consultations with the FDA regarding Roundup Ready soybean (1994), Roundup Ready canola (1995), Roundup Ready cotton (1995), Roundup Ready Corn 2 (1996), Roundup Ready sugar beet (1998), Roundup Ready Flex cotton (2005), and Roundup Ready 2 Yield soybean (2007).

b) EPSPS exerts its function in the shikimate pathway that is integral to aromatic amino acid biosynthesis in plants and microorganisms (Levin and Sprinson, 1964; Steinrücken and Amrhein, 1980). Therefore, this enzyme and its activity are found widely in food and feed derived from plant and microbial sources. Genes for numerous EPSPSs have been cloned (Padgette et al., 1996b), and the catalytic domains of this group of proteins are conserved. Bacterial EPSPSs have been well characterized with respect to their three dimensional X-ray crystal structures (Stallings et al., 1991) and detailed kinetic and chemical mechanisms (Anderson and Johnson, 1990).

c) The EPSPS from *Agrobacterium* sp. strain CP4 is highly tolerant to inhibition by glyphosate and has high catalytic efficiency, compared to most glyphosate-tolerant EPSPSs (Barry et al., 1992; Padgette et al., 1996b). The CP4 EPSPS protein thus represents one of many different EPSPSs found in nature, and the CP4 and native plant EPSPS enzymes are functionally equivalent except for their affinity to glyphosate. The CP4 EPSPS protein present in MON 87705 is similar to EPSPSs consumed in a variety of food and feed sources. CP4 EPSPS protein is homologous to EPSPSs naturally present in plants, including food crops (e.g., soybean and corn) and fungal and microbial food sources such as baker's yeast (*Saccharomyces cerevisiae*), all of which have a history of safe human consumption (Harrison et al., 1996; Padgette et al., 1996b). The similarity of the CP4 EPSPS protein to EPSPSs in a variety of foods supports extensive human consumption of the family of EPSPS proteins and the lack of health concerns. Furthermore, the ubiquitous presence of homologous EPSPS enzymes in food crops and common microorganisms establishes that EPSPS proteins, and their enzyme activity, pose no hazards for human and animal consumption.

d) The CP4 EPSPS 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 the FASTA sequence alignment tool and eight-amino acid sliding window search. An amino acid sequence may be considered to have allergenic potential if it has an exact sequence identity of at least eight linearly contiguous amino acids with a potential allergen epitope (Metcalfe, 1996 Hileman et al., 2002). Using a sliding window of less than eight amino acids can produce matches containing significant uncertainty depending on the length of the query sequence (Silvanovich et al., 2006) and are not useful to the allergy assessment process (Thomas et al., 2004).

e) The CP4 EPSPS protein is readily digestible in simulated gastric and simulated intestinal fluids (Harrison et al., 1996). Rapid degradation of the full-length CP4 EPSPS protein in SGF and SIF makes it highly unlikely for CP4 EPSPS protein to be absorbed by epithelial cells of the small intestine in a biologically active form.

f) An acute toxicology study was conducted with a CP4 EPSPS protein (Harrison et al., 1996) that was shown to be physicochemically and functionally equivalent to

the CP4 EPSPS protein produced in MON 87705. Results indicate that the CP4 EPSPS protein did not cause any adverse effects in mice, with a No Observable Adverse Effect Level (NOAEL) of 572 mg/kg, the highest dose level tested.

g) Potential human health risks from consumption of the CP4 EPSPS protein in foods derived from MON 87705 were evaluated by calculating a Margin of Exposure (MOE) between the acute mouse NOAEL for CP4 EPSPS protein 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 CP4 EPSPS protein were estimated to be 60,000 and 1,600 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 CP4 EPSPS protein produced by MON 87705.

h) Potential health risks to animals from the presence of CP4 EPSPS protein in feed were evaluated by calculating an estimate of daily dietary intake (DDI). In the worst case scenario, the percentage of the CP4 EPSPS protein consumed from MON 87705 as part of the daily protein intake for a dairy cow is 0.0907% and for both the broiler and pig is less than 0.0325%.

Using the guidance provided by the FDA, a conclusion of "no concern" is reached for the donor organism and the CP4 EPSPS protein. The food and feed products containing MON 87705 or derived from MON 87705 are as safe as soybean currently on the market for human and animal consumption.

VII. COMPOSITIONAL AND NUTRITIONAL ASSESSMENT OF MON 87705

Compositional comparisons between biotechnology-derived and conventional crops represent an integral part of a nutritional and safety assessment. Compositional assessments are performed using the principles and analytes outlined in the OECD consensus documents for soybean composition (OECD, 2001). These principles are accepted globally and have been employed previously in assessments of soybean products derived through biotechnology.

Compositional equivalence between biotechnology-derived and conventional crops provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). The OECD consensus documents emphasize quantitative measurements of essential nutrients, and known antinutrients and toxicants. This is based on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential safety and antinutritional concerns. Levels of the components in seed and forage of the biotechnology-derived crop product are compared to: 1) corresponding levels in a non-modified comparator, typically the nontransgenic parental line grown under identical conditions, and 2) natural ranges generated from an in-study evaluation of commercial varieties or from data published in the scientific literature.

MON 87705 was developed to generate soybean oil with lower levels of saturated fats (16:0 palmitic acid and 18:0 stearic acid) and higher levels of 18:1 oleic acid, with an associated decrease in 18:2 linoleic acid, through suppression of *FAD2* and *FATB* RNAs (Figure VII-1). MON 87705 contains the same major fatty acids that are found in conventional soybean, including 16:0 palmitic, 18:0 stearic, 18:1 oleic, 18:2 linoleic and 18:3 linolenic fatty acids. MON 87705 has a fatty acid profile that is comparable to other commercial high oleic vegetable oils (high oleic canola, high oleic safflower, high oleic sunflower), traditional oils, such as olive oil, that have a long-history of consumption in the diet, and canola oil that was granted GRAS status by the U.S. FDA. MON 87705 also contains the *cp4 epsps* gene encoding the CP4 EPSPS protein that is expressed throughout the plant conferring tolerance to glyphosate, the active ingredient in the Roundup family of agricultural herbicides.

Compositional analyses were conducted to assess whether the nutrient and antinutrient levels in the seed and forage derived from MON 87705 are comparable to those in the conventional soybean control, A3525, which has background genetics similar to MON 87705, but lacks the introduced traits. In addition, commercial conventional soybean varieties were included in the seed and forage composition analyses to establish a range of natural variability for each analyte, defined by a 99% tolerance interval. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods.

Seed and forage of MON 87705 and the conventional soybean control were harvested from soybean grown in three replicated plots, planted in a randomized complete block design, at each of five sites across Chile during the 2007-2008 growing season: Quilapilun, Chacabuco Province (QUI); Melipilla, Melipilla Province (MEL); Calera de Tango, Maipo Province (CdT); Rancagua, Cachapoal Province (RAN); and San

Fernando, Colchagua Province (SFR). Samples from all three replicates of MON 87705 and the control were collected from all three plots and analyzed. Four different commercial reference soybean varieties also were grown at each site for a total of 20 varieties. Samples from the commercial reference varieties grown at each site were collected from all three plots. All replicates from 19 of 20 commercial conventional reference soybean varieties were analyzed; however, one reference variety had all replicates damaged by an early frost and was excluded from the study. All MON 87705, control and reference soybean varieties were grown under normal agronomic field conditions for their respective geographic regions. Forage was collected at the R6 plant growth stage, and harvested soybean seed was collected at physiological maturity. The seed and forage collected from MON 87705, the conventional control, and the reference varieties were analyzed for compositional components.

In all, 67 analytical components were measured, 60 in seed and seven in forage. The analytes in forage included proximates (ash, fat, moisture, protein, and carbohydrates by calculation), acid detergent fiber (ADF), and neutral detergent fiber (NDF). Seed samples were analyzed for proximates, ADF, NDF, amino acids (18), fatty acids (26; C8-C24), trypsin inhibitors, phytic acid, lectin, isoflavones (daidzein, glycitein, and genistein), vitamin E, raffinose, and stachyose. Materials and methods used for compositional analysis of the seed and forage of MON 87705, the conventional soybean control, and commercial conventional reference soybean varieties are presented in Appendix E.

The composition data then were statistically compared to that of the conventional soybean control to establish substantial equivalence. Of the measured components, 17 fatty acids in seed had more than 50% of the observations below the assay limit of quantitation (LOQ) and could not be statistically analyzed. Thus, statistical analyses were conducted for 50 components (43 in seed and seven in forage). The data set was examined for evidence of biologically relevant changes using a mixed model of variance. Six sets of statistical analyses were conducted, five based on the data from each of the replicated field sites (individual-site) and the sixth analysis based on data from a combination of all five field sites (combined-site). The statistical summaries of the combined-site analysis and the individual-site analyses, reported literature and the International Life Sciences Institute-Crop Composition Database (ILSI-CCD at http://www.cropcomposition.org) ranges for the analytical components present in seed are provided in Appendix E. The compositional data set was examined for evidence of statistically significant differences ($p \leq 0.05$) between MON 87705 and the conventional soybean control. A summary of the significant differences observed between MON 87705 and the control are presented in Table VII-2.

Results of the comparisons indicate that except for the intended fatty acid changes, the composition of the seed and forage of MON 87705 is equivalent to that of the conventional soybean control A3525, in accordance with OECD guidelines. Moreover, no new fatty acids beyond those presently found in soybean were detected in MON 87705. Therefore, MON 87705 is regarded as safe and nutritious as conventional soybean for food and feed use. Further details of this assessment are provided in Section VII-A.

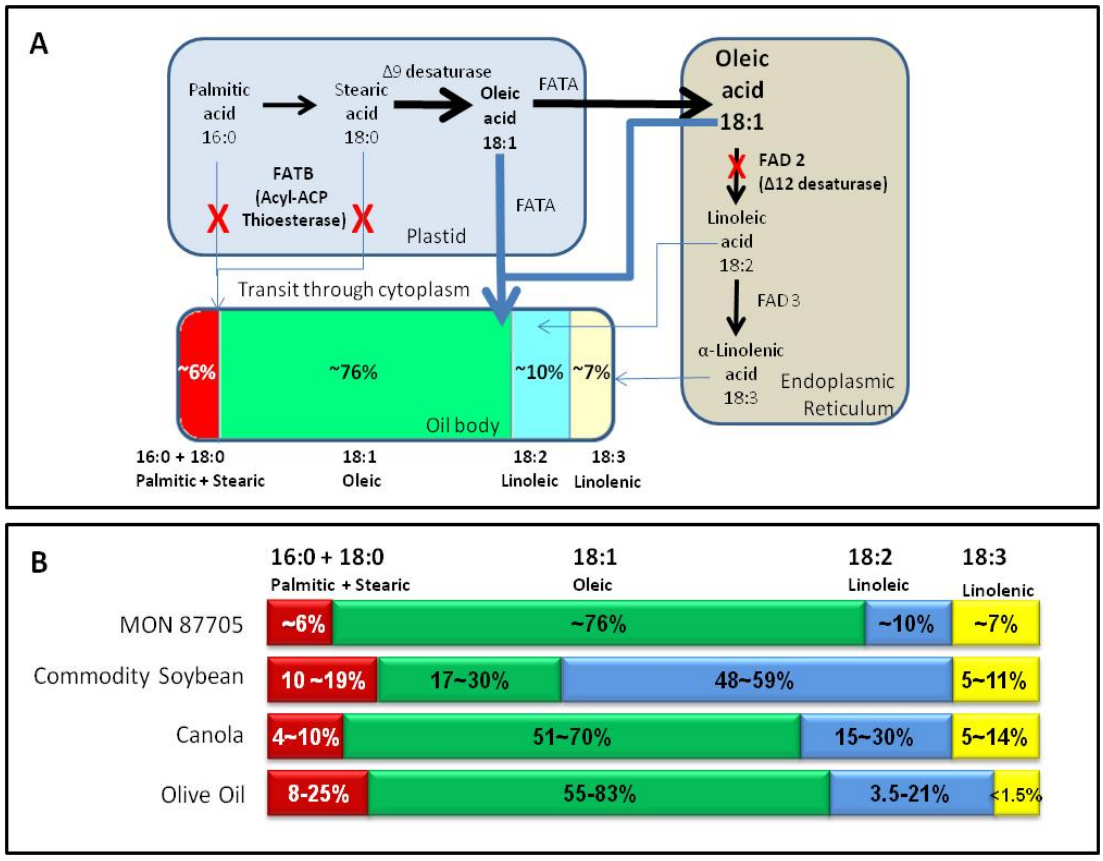


Figure VII-1. Schematic of the Soybean Fatty Acid Biosynthetic Pathway and Summary of Modified Fatty Acids in MON 87705

Panel A: Schematic of the soybean fatty acid biosynthetic pathway,

Panel B: MON 87705 soybean oil compared to commodity soybean oil and other vegetable oils

✕ Indicates suppression of endogenous FATB and FAD2 in MON 87705 seeds

VII.A. Overall Assessment of the Composition of Forage and Seed from MON 87705 Compared to the Conventional Soybean Control

Based on the comprehensive assessment procedures discussed above, MON 87705 is compositionally equivalent to conventional soybean except for the intended changes in fatty acid levels. Combined-site analysis of both forage and seed samples showed no statistically significant difference ($p>0.05$) between MON 87705 and the control for 39 of 50 comparisons. Significant differences ($p<0.05$) between MON 87705 and the conventional soybean control were detected for 10 analytes in seed (arginine, cystine, fat, 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, and 20:1 eicosenoic acid) and one analyte (ash) in forage.

The compositional analyses confirmed that MON 87705 had the intended changes in the levels of four major soybean oil fatty acids (16:0 palmitic, 18:0 stearic, 18:1 oleic and 18:2 linoleic). For the remaining seven comparisons where a significant difference ($p<0.05$) was detected, an analysis, including magnitude of the differences,

reproducibility across individual sites, and comparisons of mean analyte values to the 99% tolerance interval and literature values, indicated they were not materially different and were not biologically meaningfully different from a food and feed safety perspective. Further assessment of the statistically significant differences observed between MON 87705 and the conventional soybean control is provided in the following sections. Therefore, the compositional assessment of MON 87705 supports the conclusion that, except for intended changes in seed fatty acid composition, seed and forage produced from MON 87705 are compositionally equivalent to those of conventional soybean.

VII.A.1 Intended Changes to Fatty Acid Levels in MON 87705 Seed

As described previously, MON 87705 was developed to generate soybean oil with lower levels of saturated fats (16:0 palmitic acid and 18:0 stearic acid) and higher levels of 18:1 oleic acid, with an associated decrease in 18:2 linoleic acid, through suppression of *FATB* and *FAD2* RNAs (Figure VII-1). As expected, all of the intended changes in fatty acid levels were statistically significant in the combined-site analysis and are summarized in the Table VII-1 below.

Table VII-1. Summary of Intended Changes in Fatty Acid Levels for MON 87705 vs. the Conventional Soybean Control (A3525) in the Combined-Site Analysis

Fatty Acid (% total)	MON 87705 Mean [Range]	A3525 Mean [Range]	Commercial Tolerance Interval[2]
16:0 Palmitic[1]	2.36 [2.25 - 2.44]	10.83 [10.51 – 11.08]	[7.62, 12.55]
18:0 Stearic[1]	3.31 [3.07 - 3.82]	4.50 [4.24 – 4.85]	[2.87, 7.15]
18:1 Oleic[1]	76.47 [73.13 - 79.17]	22.81 [21.41 – 25.08]	[18.40, 30.22]
18:2 Linoleic[1]	10.10 [7.85 - 12.42]	52.86 [51.68 – 53.89]	[47.75, 56.46]

[1]Significance level = <0.001

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

The results show that the level of the saturated 16:0 palmitic acid decreased from 10.83% total FA in the conventional control to 2.36% total FA in MON 87705, and the level of the saturated 18:0 stearic acid decreased from 4.50% total FA in the conventional control to 3.31% total FA in MON 87705. Thus, total saturated fat was decreased from approximately 15.3% total FA in the conventional control to 5.7% total FA in MON 87705. In addition, the mean level of 18:1 oleic acid increased from 22.81% total FA in the conventional control to 76.47% total FA in MON 87705. These changes were associated with a decrease in the mean level of 18:2 linoleic acid from 52.86% total FA in the conventional control to 10.10% total FA in MON 87705. As expected, all intended changes in fatty acid levels in the seed of MON 87705 were statistically significant in the combined-site analysis and consistently observed at all five of the individual sites. Thus, compositional analysis confirmed MON 87705 had the intended fatty acid profile required for improved nutrition and soybean oil stability.

VII.A.2 Fatty Acid Levels in Soybean Seed

Of the 26 fatty acids analyzed in seed, 17 fatty acids had more than 50% of the observations below the assay limit of quantitation and, as a result, were excluded from the statistical analysis. Of the nine fatty acids that could be statistically analyzed, significant differences ($p<0.05$) were observed for seven fatty acids in the combined-site analysis (Table VII-2). Four of these differences were due to the intended changes in fatty acids, as described in Section VII.A.1. The three remaining significant differences

in the combined-site analysis were for 18:3 linolenic, 20:0 arachidic and 20:1 eicosenoic acids. Given the intended changes in fatty acid metabolism, these additional differences in fatty acid levels were not unexpected. The biological relevance of these differences was assessed based on the magnitude of the difference, reproducibility across sites, and comparison of mean analyte values to the 99% tolerance interval for the population of commercial conventional soybean varieties grown concurrently at the same field sites.

A combined-site statistical difference ($p<0.05$) between MON 87705 and the conventional control was observed in the levels of 18:3 linolenic acid. The decrease in linolenic acid is expected given that it is produced from 18:2 linoleic acid which was reduced by the suppression of the *FAD2* gene. Examination of the reproducibility within sites shows the levels of 18:3 linolenic acid were significantly lower than the soybean control in four of five individual-site analyses, with the absolute magnitude of the differences being small (<1.5% total FA; Appendix E). In addition, all the mean levels of 18:3 linolenic acid in MON 87705 seed from the combined-site and individual-site analyses were well within the 99% tolerance interval, and therefore these differences are not considered biologically relevant compositional changes.

Combined-site statistical differences between MON 87705 and the conventional control also were observed in levels of two minor fatty acids, 20:0 arachidic acid, and 20:1 eicosenoic acid. The mean level of 20:0 arachidic acid in MON 87705 was significantly lower than in the conventional soybean control in the combined-site analysis. An examination of the reproducibility within sites showed that the levels of 20:0 arachidic acid were consistently lower than the soybean control in all five individual-site analyses. However, the absolute magnitude of the differences was small (<0.063% total FA; Appendix E), all combined-site and individual-site means were well within the 99% tolerance interval, and therefore these differences are not considered biologically relevant compositional changes.

The mean level of 20:1 eicosenoic acid in MON 87705 was significantly higher than in the conventional soybean control in the combine-site analysis. An examination of the reproducibility within sites showed that the levels of 20:1 eicosenoic acid were consistently higher than the soybean control in all five individual-site analyses. However, the absolute magnitude of these differences was small (<0.19% total FA; Appendix E). The combined-site mean for 20:1 eicosenoic (0.18% total FA) was slightly (0.09% total FA) outside the upper end (0.25% total FA) of the 99% tolerance interval but within the values reported in ILSI-CCD. In addition, 20:1 eicosenoic acid has a history of consumption in other commonly consumed vegetable oils, such as canola (4.3% total FA), corn (0.6% total FA), mustard seed (13.0% total FA), peanut (1.7% total FA), high oleic safflower (0.5% total FA, and high oleic sunflower (0.5% total FA) (Codex, 2005). Therefore, the small change in the mean level of 20:1 eicosenoic acid in MON 87705 is not considered a biologically relevant compositional change.

These results lead to the conclusion that apart from the intended changes in the levels of four fatty acids (16:0 palmitic, 18:0 stearic, 18:1 oleic and 18:2 linoleic), the seed from MON 87705 is compositionally equivalent to conventional soybean with regard to the levels of other fatty acids. The differences observed for 18:3 linolenic, 20:0 arachidic, and 20:1 eicosenoic fatty acids were not unexpected, given the intended shift made in fatty acid metabolism (see Figure VII-1). Furthermore, the mean levels of these fatty

acids were within the 99% tolerance interval and/or the ILSI-CCD and literature values. Therefore, these differences are not considered biologically meaningful from a food and feed safety or nutritional perspective.

VII.A.3. Levels of Non-Fatty Acid Nutrients in Soybean Seed

In addition to fatty acids, soybean seed also was analyzed for the following 26 nutrients: proximates (5), ADF, NDF, amino acids (18), and vitamin E. No statistically significant differences ($p<0.05$) were observed for 23 nutrient analytes. Three analytes were statistically different ($p<0.05$) between MON 87705 and the conventional control in the combined-site analysis: total fat, arginine and cystine (Table VII-2). The biological relevance of these differences was assessed based on the magnitude of the difference, reproducibility across sites, and comparison of mean analyte values to the 99% tolerance interval for the population of commercial conventional soybean varieties grown concurrently at the same field sites. Mean analyte values were further compared to ILSI-CCD and literature ranges.

The mean level of total fat was significantly lower ($p<0.05$) in MON 87705 than the conventional soybean control in the combined-site analysis; however, the absolute magnitude of the mean difference was small (1.04% dw; Appendix E). There were no differences in total fat in any of the individual-site analyses. Furthermore, the mean level of total fat in MON 87705 was well within the 99% tolerance interval. Therefore, the difference in total fat in MON 87705 compared to the control is not considered biologically meaningful.

The mean level of cystine was significantly higher ($p<0.05$) in MON 87705 than the conventional soybean control in the combined-site analysis; however, the absolute magnitude of the mean difference was small (0.022% dw; Appendix E). There were no differences in cystine levels in any of the individual-site analyses. Furthermore, the mean level of cystine in MON 87705 was within the 99% tolerance interval. Therefore, the difference in cystine in MON 87705 compared to the control is not considered biologically meaningful.

The mean level of arginine was significantly higher ($p<0.05$) in MON 87705 than the conventional soybean control in the combined-site analysis; however, the absolute magnitude of the mean difference was small (0.1% dw). Examination of the reproducibility within sites shows that the mean level of arginine was significantly higher in only one of five individual-site analyses; however, the absolute magnitude of the mean difference was small (0.18% dw; Appendix E) These differences are not biologically relevant changes in composition, given that the mean levels of arginine in MON 87705 in the combined-site and individual-site analyses were all well within the 99% tolerance interval.

These results lead to the conclusion that the seed from MON 87705 is compositionally equivalent to conventional soybean with regard to the levels of nutrients. The differences observed for nutrients were limited in number, not consistently observed across sites, and reflect the natural variation of conventional soybean. Furthermore, the mean levels of nutrient analytes were within the 99% tolerance interval and ILSI-CCD values.

Therefore, these differences are not considered biologically meaningful from a food and feed safety or nutritional perspective.

VII.A.4. Naturally Occurring Anti-Nutrient Levels in Soybean Seed

Soybean seed contains several well-described antinutritional factors according to OECD (2001), which include: trypsin inhibitors, lectins, isoflavones (genistein, daidzein and glycitein), stachyose, raffinose, and phytic acid. Combined-site analysis of antinutrients showed no significant differences ($p>0.05$) between MON 87705 and the conventional soybean control. Additional information is provided below to complete the discussion for the group of antinutrients.

Trypsin inhibitors are heat-labile antinutrients that interfere with the digestion of proteins and result in decreased animal growth (Liener, 1994). Lectins are also heat labile, and can inhibit growth and cause death in animals if raw soybean is consumed (Liener, 1994). Both trypsin inhibitors and lectins are inactivated during processing of soybean protein products or soybean meal and, when processed appropriately, the final edible soybean fractions should contain minimal levels of these antinutrients. No significant differences ($p\geq0.05$) were observed in trypsin inhibitor levels between MON 87705 and the conventional soybean control in the combined-site or individual-site analyses.

There are three principle isoflavones in soybean seed, namely daidzein, genistein, and glycitein. Although they have been reported to possess biochemical activities, including estrogenic and anti-estrogenic effects, it is not universally accepted that the isoflavones are antinutrients because they have also been reported to have beneficial antioxidant, anticarcinogenic and heart-healthy hypocholesterolemic effects (OECD, 2001). It is well documented that isoflavone levels in soybean seed are highly variable and are greatly influenced by many factors (OECD, 2001; Messina, 2001; Nelson et al., 2001). No significant differences ($p\geq0.05$) in isoflavone levels were observed between MON 87705 and the conventional soybean control for the combined-site or individual-site analyses.

Stachyose and raffinose are low molecular weight carbohydrates present in soybean seed that are considered to be antinutrients due to their consumption, which causes flatulence. No significant differences ($p\geq0.05$) in raffinose levels were observed between MON 87705 and the conventional soybean control in the combined-site or individual-site analyses. Stachyose levels showed no differences between MON 87705 and the conventional soybean control in the combined-site analysis, but were different at one site (Table VII-2). This difference is not considered biologically relevant because it was observed only at one site and was not observed consistently across all sites.

Phytic acid present in soybean seed chelates mineral nutrients, including calcium, magnesium, potassium, iron and zinc, rendering them biologically unavailable to monogastric animals consuming the seed (Liener, 2000). Unlike trypsin inhibitors, phytic acid is not heat labile, and remains stable through most soybean processing steps. No significant differences ($p\geq0.05$) in phytic acid levels were observed between MON 87705 and the conventional soybean control for the combined-site or individual-site analyses.

Based on the data and information presented above, it is concluded that the seed from MON 87705 is compositionally equivalent to conventional soybean with regard to the levels of antinutrients.

VII.A.5. Proximate and Fiber Levels in Forage

Combined-site analysis of forage showed one significant difference ($p<0.05$) between MON 87705 and the conventional soybean control for ash. The biological relevance of this difference was assessed based on the magnitude of the difference, reproducibility across sites, and comparison of mean analyte values to the 99% tolerance interval for the population of commercial conventional soybean varieties grown concurrently at the same field sites.

The mean level of ash was significantly higher ($p<0.05$) in MON 87705 than the conventional soybean control; however, the absolute magnitude of this difference was small (0.57% dw). There were no differences in ash in any of the individual-site analyses. Furthermore, the mean level of ash in MON 87705 was well within the 99% tolerance interval. Therefore, the difference in ash in MON 87705 compared to the control is not considered biologically meaningful. These results lead to the conclusion that the forage from MON 87705 is compositionally equivalent to that from conventional soybean.

VII.B. Compositional Equivalence of MON 87705 Seed and Forage to Conventional Soybean

Consistent with OECD guidelines for soybean composition (OECD, 2001) compositional analyses were conducted to assess whether levels of nutrients, antinutrients, and key secondary metabolites in seed and forage derived from MON 87705 are comparable to those in the conventional soybean control, A3525, which has background genetics similar to MON 87705 but lacks the introduced improved fatty acid profile and glyphosate tolerance traits. Intended changes in the levels of the seed fatty acids 16:0 palmitic, 18:0 stearic, 18:1 oleic, and 18:2 linoleic comprised four of the 11 significant differences in the combined-site analyses. For the remaining seven comparisons where a significant difference ($p<0.05$) was detected, an analysis, including magnitude of differences, reproducibility across individual sites, and comparisons of mean test analyte values to the 99% tolerance interval and published values, indicates the differences are not materially different and/or not biologically meaningful from a food and feed safety or nutritional perspective. Therefore, the compositional and nutritional assessment of MON 87705 supports the conclusion that, except for intended changes in seed fatty acid composition, forage and seed produced from MON 87705 are compositionally equivalent to those of conventional soybean.

Table VII-2. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Mean Difference (MON 87705 minus Control) Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in Combined-Site Analysis						
Forage Proximate (% DW)						
Ash	8.75	8.18	6.99	0.020	[7.39 - 10.11]	[6.78, 9.91]
Seed Amino Acid (% DW)						
Arginine	2.78	2.68	3.74	0.048	[2.43 - 3.16]	[1.81, 3.62]
Cystine	0.61	0.59	3.66	0.043	[0.57 - 0.64]	[0.49, 0.69]
Seed Fatty Acid (% Total FA)						
16:0 Palmitic	2.36	10.83	-78.18	<0.001	[2.25 - 2.44]	[7.62, 12.55]
18:0 Stearic	3.31	4.50	-26.39	<0.001	[3.07 - 3.82]	[2.87, 7.15]
18:1 Oleic	76.47	22.81	235.20	<0.001	[73.13 - 79.17]	[18.40, 30.22]
18:2 Linoleic	10.10	52.86	-80.90	<0.001	[7.85 - 12.42]	[47.75, 56.46]

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in Combined-Site Analysis						
Seed Fatty Acid (% Total FA)						
18:3 Linolenic	6.69	8.02	-16.59	<0.001	[5.55 - 7.81]	[4.97, 9.93]
20:0 Arachidic	0.30	0.34	-11.72	0.005	[0.28 - 0.36]	[0.22, 0.53]
20:1 Eicosenoic	0.34	0.19	79.85	<0.001	[0.27 - 0.40]	[0.13, 0.25]
Seed Proximate (% DW)						
Total Fat	18.29	19.33	-5.38	<0.001	[16.55 - 19.50]	[15.35, 25.95]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Site CdT	2.31	10.80	-78.62	<0.001	[2.29 - 2.32]	[7.62, 12.55]
16:0 Palmitic Site MEL	2.39	10.83	-77.92	<0.001	[2.35 - 2.42]	
16:0 Palmitic Site QUI	2.30	10.56	-78.24	0.005	[2.25 - 2.37]	

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Site RAN	2.40	10.96	-78.12	<0.001	[2.39 - 2.40]	[7.62, 12.55]
16:0 Palmitic Site SFR	2.42	11.00	-78.00	<0.001	[2.40 - 2.44]	
18:0 Stearic Site CdT	3.17	4.58	-30.88	<0.001	[3.09 - 3.23]	[2.87, 7.15]
18:0 Stearic Site MEL	3.33	4.39	-24.06	0.018	[3.20 - 3.47]	
18:0 Stearic Site QUI	3.51	4.82	-27.20	0.004	[3.15 - 3.82]	
18:0 Stearic Site RAN	3.34	4.50	-25.73	0.001	[3.28 - 3.41]	
18:0 Stearic Site SFR	3.22	4.31	-25.26	0.001	[3.07 - 3.41]	

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:1 Oleic Site CdT	76.44	23.02	232.08	<0.001	[76.35 - 76.60]	[18.40, 30.22]
18:1 Oleic Site MEL	76.10	22.31	241.09	<0.001	[75.68 - 76.33]	
18:1 Oleic Site QUI	78.61	24.95	215.05	0.003	[77.70 - 79.17]	
18:1 Oleic Site RAN	74.69	21.53	246.87	<0.001	[73.13 - 75.98]	
18:1 Oleic Site SFR	76.49	22.42	241.12	<0.001	[75.33 - 77.21]	
18:2 Linoleic Site CdT	10.09	52.43	-80.75	<0.001	[9.94 - 10.22]	[47.75, 56.46]
18:2 Linoleic Site MEL	10.50	53.48	-80.38	<0.001	[10.16 - 10.92]	

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:2 Linoleic Site QUI	8.75	51.70	-83.07	0.006	[7.85 - 10.02]	[47.75, 56.46]
18:2 Linoleic Site RAN	11.32	53.73	-78.92	<0.001	[10.37 - 12.42]	
18:2 Linoleic Site SFR	9.82	52.84	-81.42	<0.001	[9.33 - 10.55]	
18:3 Linolenic Site CdT	6.90	8.15	-15.32	0.001	[6.85 - 6.94]	[4.97, 9.93]
18:3 Linolenic Site MEL	6.58	8.00	-17.72	0.002	[6.53 - 6.65]	
18:3 Linolenic Site QUI	5.64	7.02	-19.69	0.029	[5.55 - 5.71]	
18:3 Linolenic Site SFR	6.98	8.49	-17.72	0.009	[6.79 - 7.26]	

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
20:0 Arachidic Site CdT	0.29	0.35	-18.10	0.016	[0.28 - 0.29]	[0.22, 0.53]
20:0 Arachidic Site MEL	0.30	0.34	-11.86	0.026	[0.29 - 0.30]	
20:0 Arachidic Site QUI	0.33	0.36	-8.84	0.041	[0.30 - 0.36]	
20:0 Arachidic Site RAN	0.28	0.33	-13.08	0.014	[0.28 - 0.29]	
20:0 Arachidic Site SFR	0.29	0.32	-8.18	0.006	[0.29 - 0.29]	
20:1 Eicosenoic Site CdT	0.36	0.21	76.81	<0.001	[0.36 - 0.38]	[0.13, 0.25]
20:1 Eicosenoic Site MEL	0.35	0.20	70.85	0.001	[0.34 - 0.36]	

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
20:1 Eicosenoic Site QUI	0.38	0.20	89.53	0.049	[0.37 - 0.40]	[0.13, 0.25]
20:1 Eicosenoic Site RAN	0.29	0.16	82.18	0.003	[0.27 - 0.31]	
20:1 Eicosenoic Site SFR	0.33	0.18	80.72	0.005	[0.32 - 0.35]	
Seed Fiber (% DW)						
Acid Detergent Fiber Site CdT	18.23	16.27	12.10	0.049	[17.57 - 18.58]	[12.71, 19.29]
Acid Detergent Fiber Site RAN	16.32	13.94	17.07	0.002	[15.71 - 16.78]	
Statistical Differences Observed in One Site						
Forage Proximate (% DW)						
Carbohydrates Site RAN	69.77	72.09	-3.22	0.027	[68.94 - 71.06]	[64.45, 80.50]
Total Fat Site MEL	5.79	6.76	-14.29	0.030	[5.37 - 6.57]	[0, 9.74]

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in One Site						
Seed Amino Acid (% DW)						
Alanine Site SFR	1.51	1.44	4.62	0.024	[1.49 - 1.54]	[1.25, 1.92]
Arginine Site SFR	2.52	2.34	7.56	0.047	[2.43 - 2.64]	[1.81, 3.62]
Aspartic Acid Site SFR	3.76	3.56	5.48	0.009	[3.67 - 3.88]	[3.02, 5.11]
Glutamic Acid Site SFR	5.90	5.53	6.62	0.008	[5.72 - 6.12]	[4.42, 8.48]
Histidine Site SFR	0.90	0.85	5.90	0.018	[0.88 - 0.94]	[0.74, 1.16]
Leucine Site SFR	2.54	2.41	5.12	0.014	[2.47 - 2.61]	[2.06, 3.41]
Lysine Site SFR	2.25	2.13	5.32	0.007	[2.19 - 2.30]	[1.87, 2.81]

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in One Site						
Seed Amino Acid (% DW)						
Phenylalanine Site SFR	1.68	1.60	4.83	0.019	[1.64 - 1.73]	[1.35, 2.31]
Proline Site SFR	1.62	1.55	5.02	0.021	[1.59 - 1.66]	[1.29, 2.21]
Tyrosine Site SFR	1.18	1.12	5.72	0.042	[1.17 - 1.20]	[0.99, 1.49]
Seed Fatty Acid (% Total FA)						
24:0 Lignoceric Site CdT	0.15	0.15	-3.24	0.008	[0.14 - 0.15]	[0.030, 0.26]
Seed Fiber (% DW)						
Neutral Detergent Fiber Site CdT	21.04	17.99	16.97	0.009	[20.47 - 22.18]	[12.07, 21.51]
Seed Proximate (% DW)						
Carbohydrates Site CdT	41.82	40.05	4.40	0.016	[41.62 - 42.00]	[30.78, 45.86]
Seed Vitamin (mg/100g DW)						
Vitamin E Site MEL	3.26	3.83	-15.05	0.005	[3.15 - 3.45]	[0, 7.36]

Table VII-2 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Significance (p-Value)	MON 87705 Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in One Site						
Seed Antinutrient (% DW)						
Stachyose Site CdT	3.76	3.10	21.27	0.046	[3.55 - 4.16]	[1.96, 4.41]

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

VII.C. Compositional Comparison of Processed Fractions from Soybean Seed of MON 87705 and the Conventional Control

To prepare soybean processed fractions, seed samples were collected from field trials conducted with MON 87705 and the conventional soybean control at two field sites (Jefferson County, IA and Clinton County, IL) in the U.S. during the 2007 growing season. In addition, 12 commercial conventional soybean varieties were grown at three field sites in the U.S. and processed to determine a 99% tolerance interval for each component analyzed. The seed samples were processed into defatted toasted soybean meal (TD soybean meal); refined, bleached, and deodorized soybean oil (RBD oil); protein isolate; and crude lecithin fractions. The processed fractions were analyzed according to the principles outlined in the OECD consensus document for soybean composition (OECD, 2001). The TD soybean meal was analyzed for proximates (moisture, protein, fat, ash, and carbohydrates by calculation), ADF, NDF, amino acids, trypsin inhibitors and phytic acid. The RBD oil was analyzed for fatty acids and vitamin E (α-tocopherol). The protein isolate fraction was analyzed for amino acids and moisture. The crude lecithin fraction was analyzed for phosphatides (α-phosphatidic acid, α-phosphatidylcholine, α-phosphatidylethanolamine, and α-phosphatidylinositiol). Compositional analyses were conducted to assess whether the processed fractions from MON 87705 are comparable to those of the conventional soybean control, A3525, which has background genetics similar to MON 87705, but lacks the introduced improved fatty acid profile and glyphosate tolerance traits. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods. The statistical analysis compared MON 87705 and the conventional control across the two sites (combined-site). Statistical summary of the composition of each processed fraction and summary of the significant differences observed between the processed fractions prepared from the seed of MON 87705 and the conventional control are included in Appendix E.

Results show that there were no statistically significant differences ($p>0.05$) between MON 87705 and the conventional control for components measured in the protein isolate fraction or phosphatides of crude lecithin. Comparison of the composition of TD soybean meal processed from MON 87705 and the conventional soybean control showed no differences ($p>0.05$) for 21 of the 27 components analyzed. Significant differences ($p<0.05$) were observed for six components of the TD soybean meal: alanine, glycine, isoleucine, lysine, valine, and NDF. The absolute magnitude of the differences was small (<1.8% dw) and the MON 87705 mean values fell within the 99% tolerance interval for the conventional soybean varieties and also within the range of published values for conventional soybean. The low levels of residual oil (0.78% dw, as total fat) present in the TD soybean meal from MON 87705 also are expected to reflect the intended changes in fatty acid levels observed in seed.

As expected, and consistent with the results obtained for seed fatty acid levels, the intended fatty acid changes (16:0 palmitic, 18:0 palmitic, 18:1 oleic and 18:2 linoleic) also were observed in RBD oil. In addition, six fatty acids were detected in RBD oil that were not detected in seed: 14:0 myristic acid, 16:1 palmitoleic acid, 17:0 margaric (heptadecanoic) acid, 17:1 9c heptadecenoic acid, 18:2 other *trans* isomer fatty acids

(excluding 9t,12t linolelaidic), and 18:2 6c,9c, octadecadienoic acid. As observed in seed, levels of several less abundant fatty acids were significantly different ($p<0.05$) between the RBD oil from MON 87705 and the conventional control. Differences were observed for 14:0 myristic acid, 16:1 palmitoleic acid, 17:0 margaric (heptadecanoic) acid, 20:0 arachidic, 20:1 eicosenoic and 22:0 behenic acids. However, the absolute magnitude of the differences was small (<0.15% total FA), and the MON 87705 mean values fell within the 99% tolerance intervals for the reference varieties and/or within published ranges for conventional soybean oil (Codex, 2005; Appendix E).

A significant increase ($p<0.05$) in the level of the minor fatty acid 17:1 9c heptadecenoic acid was observed in MON 87705 compared to conventional control RBD oil. This is not unexpected, given the intended shift in fatty acid levels in MON 87705. The mean level of 17:1 9c heptadecenoic acid in MON 87705 (0.12% total FA) was outside the range of values obtained for the RBD oil from commercial references. However, 17:1 9c heptadecenoic acid is present at similar or higher levels in a variety of oils (canola, corn, peanut, high oleic safflower, and high oleic sunflower; Codex, 2005) and foods (tofu, ground beef, and soft-spread margarine; USDA-ARS, 2007). Therefore, there are no adverse food and feed safety or nutrition effects associated with the levels of 17:1 9c heptadecenoic acid observed in MON 87705 soybean oil. The remaining minor fatty acids 18:2 other *trans* (excluding linolelaidic) and 18:2 6c,9c, octadecadienoic acid, not detected in seed and are believed to arise from the spontaneous isomerization of unsaturated fatty acids during the oil refining process. Levels of these fatty acids were significantly lower ($p<0.05$) in MON 87705 compared to control RBD oil and thus, these differences were not considered biologically relevant from a food and feed safety or nutritional perspective (Chardigny et al., 1996).

Therefore, this supports the conclusion that, except for the intended changes in fatty acid composition, minor differences in the levels of less abundant fatty acids and occurrence of low levels of minor fatty acids due to spontaneous isomerisation during the oil refining process, the processed fractions produced from MON 87705 are compositionally equivalent to those of conventional soybean.

VII.D Safety and Nutritional Assessment of the Intended Changes in MON 87705

MON 87705 was developed to generate soybean oil with decreased levels of saturated fats (16:0 palmitic acid and 18:0 stearic acid) and increased levels of 18:1 oleic acid, with an associated decrease in 18:2 linoleic acid. Replacement of conventional soybean oil with MON 87705 soybean oil under the proposed food uses results in changes in the fatty acid composition in the U.S. diet that lead to higher oleic acid intake,and lower consumption of saturated fats (i.e., 16:0 palmitic and 18:0 linoleic acid) with no impact on total fat intake. This assessment assumes all of the targeted oil components of the foods proposed for replacement that are consumed in the U.S. are replaced with MON 87705 soybean oil. Therefore, the results presented in this petition represent a theoretical maximal effect of MON 87705 soybean oil on fatty acid composition of the diet. The nutritional impact from the use of MON 87705 soybean oil in targeted foods under the intended conditions of use is estimated to result in changes in fatty acid consumption that are within current dietary guidelines for fatty acid intake (Lichtenstein et al., 2006; USDA-ERS 2005; WHO/FAO, 2003). A discussion of the safety and

nutritional impact resulting from the intended changes in MON 87705 is included in Appendix M.

VII.E Safety and Nutrition Assessment Conclusion

In conclusion, except for the intended changes in fatty acid levels, the compositional equivalence of MON 87705 seed, forage, and processed fractions to conventional soybean has been demonstrated in accordance with OECD guidelines. In addition, the nutritional impact from the use of MON 87705 soybean oil in targeted foods under the intended conditions of use is estimated to result in changes in fatty acid consumption that are within current dietary guidelines for fatty acid intake. Therefore, MON 87705 is regarded to be as safe and nutritious as conventional soybean for food and feed use.

VIII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides an evaluation of the phenotypic and agronomic characteristics and environmental interactions of MON 87705 compared to the conventional A3525 control, a conventional soybean variety that has background genetics similar to MON 87705 but does not possess the improved fatty acid profile and glyphosate tolerance trait. Phenotypic and agronomic characteristics of MON 87705 were evaluated in a comparative manner to assess plant pest potential (OECD, 1993). In the phenotypic, agronomic, and environmental interactions assessment of MON 87705, 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 87705 plant characterization and environmental interactions data cover six general categories: 1) germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive growth (including pollen characteristics); 4) seed retention on the plant and lodging; 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.

Results from the phenotypic and agronomic assessments indicate that MON 87705 does not possess characteristics that would confer a plant pest risk or significant environmental impact compared to conventional soybean. Data on environmental interactions also indicate that MON 87705 does not confer any increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors.

VIII.A. Characteristics Measured for Assessment

The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity (OECD, 1993) and were comprised of a combination of field, greenhouse, and laboratory studies conducted by scientists who are familiar with the production and evaluation of soybean. In each of these assessments, MON 87705 was compared to an appropriate conventional control that had a genetic background similar to MON 87705 but did not possess the improved fatty acid profile and glyphosate tolerance trait. In addition, multiple commercial soybean varieties (see Appendix F and Tables F-1, G-1, and I-1) were included to provide a range of comparative values that are representative of existing commercial soybean varieties for each measured phenotypic, agronomic, and environmental interaction characteristic. Data collected from the commercial reference varieties reflect a range of selection and breeding for desirable characteristics and therefore can provide context for interpreting experimental results.

Table VIII-1. Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials or Greenhouse Studies

Data Category	Characteristics measured	Evaluation timing[1]	Evaluation description (measurement endpoints)
Germination, dormancy, and emergence	Normal germinated	Day 5 and 8 (20/30°C)	% of seed producing seedlings exhibiting normal developmental characteristics
	Abnormal germinated	Day 8 (20/30°C)	% of seed that could not be classified as normal germinated
	Germinated	Day 5, 8, and 13 (10, 20, 30, 10/20 and 10/30°C)	% of seed that had germinated normally and abnormally
	Dead	Day 5 and 8 (10, 20, 30, 10/20, 10/30, and 20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C)	% of seeds that had visibly deteriorated and had become soft to the touch
	Hard viable and nonviable	Day 8 (20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C)	% of seeds that did not imbibe water and remained hard to the touch
	Firm swollen viable and nonviable	Day 8 (20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C)	% of seeds that imbibed water
	Early stand count	V2 - V4	Number of emerged plants in two rows, standardized to 20 ft rows
	Final stand count	Maturity, R8	Number of plants in two rows, standardized to 20 ft rows
Vegetative growth	Seedling vigor	V2 - V4	Rated on a 1-9 scale, where 1-3 = excellent, 4-6 = average, and 7-9 = poor vigor
	Growth stage assessment	Every two-three weeks, V2-R8	Average soybean plant growth stage per plot
	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
Reproductive growth	Days to 50% flowering	Flowering, R1-R2	Calendar day number when approximately 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
	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
Seed retention and lodging	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
Plant-symbiont interactions	Biomass	6 weeks after emergence in greenhouse	Nodule, root, and shoot dry weight
	Nodule number	6 weeks after emergence in greenhouse	Nodule number
	Total nitrogen	6 weeks after emergence in greenhouse	Shoot total nitrogen (% and g/plant)

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

Data Category	Characteristics measured	Evaluation timing[1]	Evaluation description (measurement endpoints)
Plant interactions	Plant response to abiotic stressors and disease damage	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
	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 of pest and beneficial arthropods

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

VIII.B. Interpretation of Phenotypic and Environmental Interaction Data

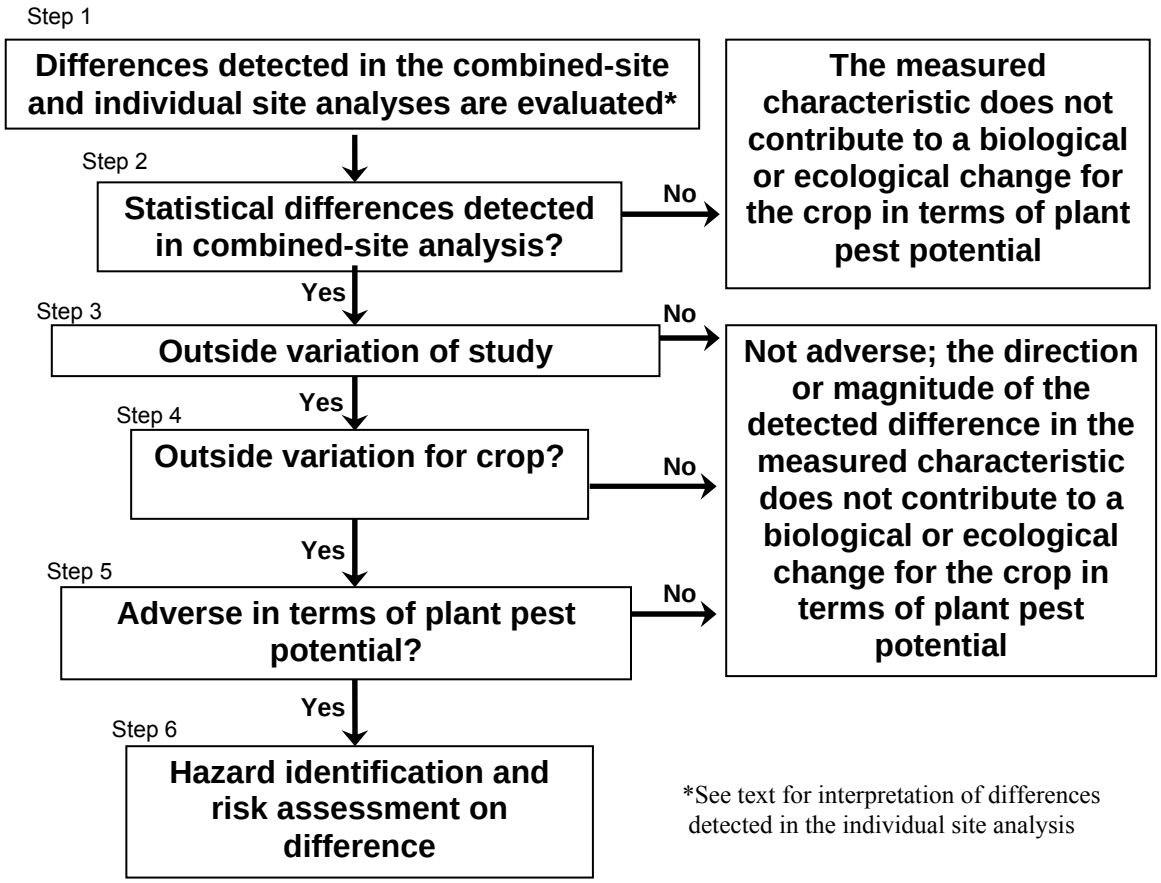
Plant pest risk assessments for biotechnology-derived crops are, by standard, comparative assessments. Familiarity provides a basis from which the potential environmental impact of a biotechnology-derived plant can be evaluated. The concept of familiarity is based on the fact that the biotechnology-derived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

Expert knowledge and experience with conventionally bred soybean was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for soybean. As such, 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., seed dormancy, pre-harvest seed loss characteristics, and lodging) was used to assess whether there was an increase in weediness potential, an element of APHIS's plant pest determination. 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.

Experienced scientists familiar with each experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Study personnel assessed that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes, and for possible evidence of an unexpected plant response. These scientists did not identify any unexpected observations or issues in the course of these evaluations. Data were then subjected to 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 a step-wise method (Figure VIII-1 or a similar method). All detected differences for a characteristic are considered in the context of whether or not the difference would increase the plant pest potential of the biotechnology-derived crop. Ultimately, a weight of evidence approach considering all characteristics and 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 pest 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 Agronomic and Phenotypic Data Interpretation Methods

- **Steps 1 and 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 whether or not it is adverse in terms of plant pest potential.
- **Step 6. Conduct Risk Assessment on Identified Hazard.** If an adverse effect (hazard) is identified, risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced plant pest potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for and effects of trait transfer to feral populations of the crop or to a sexually compatible species.

VIII.D. Phenotypic, Agronomic and Environmental Interactions Characteristics

As a significant part of the evaluation of MON 87705, 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 87705, data were collected that address specific environmental risks regarding plant pest potential based on the considerations of USDA-APHIS.

VIII.D.1. Seed Dormancy and Germination Characteristic

APHIS considers the potential for weediness to constitute a plant pest factor (CFR § 340.6). Seed germination and dormancy mechanisms vary with species and their genetic basis tends to be complex. Seed dormancy (e.g., hard seed) is an important characteristic that is often associated with plants that are considered as weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003), where in soybean it is not uncommon to observe low levels of hard seed (Mullin and Xu, 2001; Potts et al., 1978). Standardized germination assays are available and routinely used to measure the germination characteristics of soybean seed. The Association of Official Seed Analysts, an internationally recognized seed testing organization, recommends a temperature range of 20/30° C as optimal for germination of soybean (AOSA, 2007).

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 87705 and A3525, where A3525 served as a comparable control

because it has background genetics similar to MON 87705 but does not possess the improved fatty acid profile and glyphosate tolerance trait. In addition, eight commercially available soybean varieties were included as references to provide baseline values common to soybean. The seed lots for MON 87705, the conventional soybean control and reference varieties were produced during 2007 in Iowa (IA), Indiana (IN), and Missouri (MO), geographic areas which represent environmentally relevant conditions for soybean production for this product. In addition to the AOSA recommended temperature range of 20/30 °C, seed was tested at five other temperature regimes of 10, 20, 30, 10/20, and 10/30 °C to assess seed germination properties. The details of the materials, experimental methods, and germination data from all individual production sites are presented in Appendix F.

No statistically significant differences were detected between MON 87705 and the control for percent viable hard seed in any temperature regime with the exception of the 20 °C temperature regime (Table VIII-2). At 20 °C, MON 87705 had lower percent viable hard seed than the control (0.0 vs. 0.3%). The mean value for percent viable hard seed of MON 87705 was within the reference range (0.0 – 0.3%). Thus, the statistical difference detected for percent hard seed is unlikely to be biologically meaningful in terms of increased weed potential of MON 87705 compared to conventional soybean. Furthermore, a decrease in hard seed would not contribute to increased weediness of soybean.

No statistically significant differences were detected between MON 87705 and the control for percent viable firm swollen seed in any temperature regime (Table VIII-2). Within some temperature regimes, it was not possible to conduct an analysis of variance for percent viable firm swollen seed because there was no variability present in the data. For these data, the values for MON 87705 and the control were all zero, indicating no biological differences. Additionally, no statistically significant differences were detected between MON 87705 and the control for percent germinated, viable hard, dead, or viable firm swollen seed in the 10, 10/20, or 10/30 °C temperature regimes.

Three other statistically significant differences were detected between MON 87705 and the control in the combined-site analysis (Table VIII-2). MON 87705 had lower percent germinated seed than the control at 30 °C (92.8 vs. 95.5%), and had higher percent dead seed than the control at 30 °C (7.3 vs. 4.5%) and 20/30 ºC (2.7 vs. 1.3%). Lower percent germinated seed and higher percent dead seed would not contribute to increased weediness. Furthermore, all values were well within the recommended standards for certified soybean seed (AOSCA, 2009b).

The biological characteristics evaluated in this study were used to characterize MON 87705 in the context of plant pest risk assessment. Based on the dormancy and germination characteristics assessed, the results of this study, particularly the lack of increased hard seed, demonstrate there were no changes indicative of increased weed potential of MON 87705 relative to conventional soybean.

Table VIII-2. Germination Characteristics of MON 87705 and A3525

Temp. Regime [1]	Category	Mean % (S.E.)[2]		
		MON 87705	A3525	Reference Range [3]
10 °C	Germinated	97.7 (0.7)	98.4 (0.4)	98.2 – 99.5
	Viable Hard	0.1 (0.1)	0.3 (0.1)	0.0 – 0.8
	Dead	2.1 (0.8)	1.1 (0.4)	0.3 – 1.3
	Viable Firm-Swollen	0.2 (0.1)	0.3 (0.1)	0.0 – 0.5
20 °C	Germinated	99.1 (0.5)	98.8 (0.3)	96.9 – 99.8
	Viable Hard	0.0 (0.0) *	0.3 (0.1)	0.0 – 0.3
	Dead	0.9 (0.5)	0.8 (0.3)	0.3 – 3.1
	Viable Firm-Swollen	0.0 (0.0)†	0.0 (0.0)	0.0 – 0.0
30 °C	Germinated	92.8 (2.4)*	95.5 (0.6)	94.4 – 99.5
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
	Dead	7.3 (2.4) *	4.5 (0.6)	0.3 – 5.4
	Viable Firm-Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0
10/20 °C	Germinated	99.3 (0.2)	99.6 (0.2)	98.4 – 100.0
	Viable Hard	0.1 (0.1)	0.1 (0.1)	0.0 – 0.3
	Dead	0.6 (0.1)	0.3 (0.2)	0.0 – 1.6
	Viable Firm-Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0
10/30 °C	Germinated	98.0 (0.7)	98.8 (0.4)	98.0 – 99.5
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
	Dead	2.0 (0.7)	1.3 (0.4)	0.5 – 1.8
	Viable Firm-Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0
20/30 °C (AOSA)	Normal Germinated	92.4 (1.0)	93.6 (0.7)	57.0 – 98.5
	Abnormal Germinated	4.9 (0.7)	5.0 (0.7)	1.3 – 42.5
	Viable Hard	0.0 (0.0)	0.2 (0.1)	0.0 – 0.4
	Dead	2.7 (0.8) *	1.3 (0.4)	0.3 – 2.4
	Viable Firm-Swollen	0.0 (0.0)	0.0 (0.0)	0.0 – 0.1

Note: The data were analyzed according to three randomized complete block (RCB) designs with four replications; each site represented a separate RCB.

*Indicates a statistically significant difference between MON 87705 and the control ($p < 0.05$).

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

[1] For the alternating temperature regimes of 10/20, 10/30, or 20/30 °C, the lower temperature was maintained for 16 hours, and the higher temperature for eight hours.

[2] Means based on four replicates ($n = 4$) of approximately 100 seeds. In some instances, the total percentage for both MON 87705 and the control did not equal exactly 100% due to numerical rounding of the means. S.E. = Standard Error.

[3] Minimum and maximum means determined from among the eight commercially available reference soybean varieties produced at the sites.

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 87705. These data were developed to provide APHIS with a detailed characterization of MON 87705 relative to the conventional soybean control, A3525, and commercially available soybean. According to CFR § 340.6, as part of the petition to seek deregulation, a petitioner must submit "A detailed description of the phenotype of the regulated article." This information is being provided to assess whether there are phenotypic differences between MON 87705 compared to the unmodified recipient organism that may impact its pest potential. Environmental interactions were also assessed as an indirect indicator of phenotypic changes to MON 87705 relative to the same comparators described above. The purpose of these field evaluations was to assess the phenotypic and agronomic characteristics and the plant-insect, plant-disease, or plant-abiotic stressor interactions of MON 87705 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 87705 has enhanced plant pest potential.

Data were collected at 17 field locations during 2007 to thoroughly evaluate phenotypic, agronomic, and environmental interaction characteristics. These 17 locations provided a diverse range of environmental and agronomic conditions representative of commercial soybean production areas in the U.S. (Table VIII-3). The experiments were arranged as randomized complete block designs. The categories and timings of phenotypic characteristics and environmental interactions evaluated are included in Table VIII-1. The methods and detailed results of the individual site data comparisons are presented and discussed in Appendix G, while the combined-site analyses are summarized below. The results of this assessment showed the improved fatty acid profile and glyphosate tolerance trait did not unexpectedly alter MON 87705 compared to conventional soybean in terms of weediness potential, and the lack of differences in plant response to abiotic stressors, disease damage, arthropod damage, and arthropod pest and beneficial insect abundance further support the conclusion that the introduction of the modified oil profile trait is unlikely to increase plant pest potential.

VIII.D.2.1. Field Phenotypic and Agronomic Characteristics

A total of 14 phenotypic and agronomic characteristics were evaluated. For the combined-site analyses, no significant differences were detected between MON 87705 and the control for seedling vigor, plant height, lodging, pod shattering, seed moisture, test weight, or yield (Table VIII-4). Four statistically significant differences were detected between MON 87705 and the control in the combined site analysis. MON 87705 was lower than the control for both early stand count (235.1 vs. 256.1 plants/plot) and final stand count (216.3 vs. 239.3 plants/plot); however, mean counts of MON 87705 were within the reference soybean varieties range for both stand count evaluations. Furthermore, it is unlikely that decreased stand counts would contribute to increased weed potential of MON 87705 and the detected differences in stand count did not impact final yield. MON 87705 flowered approximately one day later than the

control (198.1 vs. 196.9 days after 1 Jan. 2007) and the weight of 100 seeds was lower for MON 87705 compared to the control (15.6 vs. 16.1 g). The differences in days to 50% flowering and 100 seed weight were relatively small in magnitude (0.6% and 3.2%, respectively), and the mean values of MON 87705 were within the range of the reference soybean varieties for both characteristics. Thus, the differences in days to 50% flowering and 100 seed weight are unlikely to be biologically meaningful in terms of increased weed potential.

Flower color, plant pubescence, and plant growth stage data were categorical and were not statistically analyzed; however, at each site all plants of MON 87705 and the control had purple flowers and pubescence as expected. Furthermore, MON 87705 and the control were within the same range of plant growth stages for 113 out of the 114 growth stage observations among the sites. During a single observation at one site, MON 87705 plants were at the V6 growth stage while the control plants were at V7; however, the growth stage of MON 87705 was within the range of growth stages observed for the reference soybean varieties (Appendix G; Table G-4).

The phenotypic and agronomic characteristics evaluated in this study were used to provide a detailed description of MON 87705 compared to the nontransformed control (A3525). A subset of these characteristics was useful to assess the weediness potential of MON 87705 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 87705 compared to the conventional soybean control.

Table VIII-3. Field Phenotypic Evaluation Sites for MON 87705 during 2007

Location†	Location Code	USDA-APHIS Notification Number
Jackson County, Arkansas *	AR	07-043-105n 07-115-103n
Jefferson County, Iowa	IA1	07-043-104n
Benton County, Iowa	IA2	07-043-104n
Clinton County, Illinois	IL1	07-043-104n
Stark County, Illinois	IL2	07-043-104n
Warren County, Illinois	IL3	07-043-105n
Boone County, Indiana	IN1	07-043-104n
Parke County, Indiana	IN2	07-043-105n
Pawnee County, Kansas	KS	07-043-105n
Ottawa County, Michigan	MI	07-043-105n
Lincoln County, Missouri	MO2	07-043-105n
St. Louis County, Missouri	MO3	07-043-105n
Macon County, Missouri	MO4	07-043-105n
York County, Nebraska	NE	07-043-104n
Fayette County, Ohio	OH	07-043-104n
Berks County, Pennsylvania	PA	07-043-104n
Walworth County, Wisconsin	WI	07-043-104n

* The Arkansas site utilized two USDA-APHIS notifications. 07-043-105n was a release notification and 07-115-103n was a movement notification.
†Shelby County, Missouri (USDA-APHIS notification number 07-043-105n) data not reported due to wild animal damage early in the season.

Table VIII-4. Plant Growth and Development Data across 17 Locations during 2007

	MON 87705*	A3525	Reference Range[1]	
Phenotypic Characteristic (units)	**Mean (S.E.)**	**Mean (S.E.)**	**Minimum**	**Maximum**
Early stand count (#/plot)	235.1* (8.8)	256.1 (6.9)	142.3	267.2
Seedling vigor (1-9 scale)	3.0 (0.2)	2.9 (0.2)	2.3	5.4
Days to 50% flowering[2]	198.1* (1.1)	196.9 (1.1)	194.5	200.1
Plant height (in)	34.2 (0.9)	35.1 (0.9)	26.6	43.0
Lodging (0-9 scale)	1.8 (0.3)	1.6 (0.3)	0.4	2.3
Pod shattering (0-9 scale)	0.2 (0.1)	0.2 (0.1)	0.0	0.5
Final stand count (#/plot)	216.3* (8.7)	239.3 (7.3)	145.0	258.2
Seed moisture (%)	12.3 (0.3)	12.3 (0.3)	11.0	13.6
100 seed weight (g)	15.6* (0.4)	16.1 (0.4)	13.8	20.5
Test weight (lb/bu)	54.3 (0.6)	54.0 (0.7)	51.7	56.0
Yield (bu/ac)	54.3 (2.5)	54.8 (2.3)	38.7	63.8

* Indicates a statistically significant difference between MON 87705 and the conventional soybean control (A3525) ($p < 0.05$).
[1] Reference range = Minimum and maximum mean values among the 17 commercially available reference soybean varieties.
[2] Calendar day number when approximately 50% of the plants in each plot were flowering.
S.E. = standard error. Means based on n = 51 (except 100 seed weight where n = 48).

VIII.D.2.2. Environmental Interaction Analyses

APHIS considers the environmental interaction potential of the biotechnology-derived soybean compared to its conventional counterpart to determine the potential for increased weedy or invasive characteristics. Evaluations of environmental interactions were conducted as part of the plant characterization for MON 87705. In the 2007 U.S. field trials conducted for evaluation of phenotypic and agronomic characteristics of MON 87705, 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, G-8, and G-9, respectively) also were collected. These data are used as part of the environmental risk assessment to assess the plant pest potential and potential increased adverse impact on NTOs for MON 87705 compared to the conventional soybean control (see Section X and Section XI for additional discussion). In addition, multiple commercial soybean varieties were included in the analysis to establish a range of natural variability for each assessed characteristic. The environmental interactions evaluation included data collected in the phenotypic studies (plant-insect, plant-disease, and plant-environment interactions). The results of this assessment showed the improved fatty acid profile and glyphosate tolerance trait did not unexpectedly alter MON 87705 compared to conventional soybean in terms of pest potential. 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 improved fatty acid profile and glyphosate tolerance 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 all 17 sites, and arthropod abundance was assessed from collections performed three times during the growing season at four of the 17 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 at 17 sites, and arthropod abundance data were collected quantitatively from four sites. 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 87705 and the control were considered qualitatively different in response to a stressor if the ratings between MON 87705 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 soybean for each stressor assessed.

In an assessment of abiotic stress response, disease damage, and arthropod damage, no differences were detected between MON 87705 and the conventional soybean control for 574 of 579 comparisons (including 167 abiotic stress response, 206 disease damage, and 206 arthropod damage comparisons) among all observations at the 17 sites (Appendix G; Tables G-5, G-6, and G-7). The five observed differences were in the disease and arthropod damage categories. MON 87705 had less damage than the control from

bacterial blight during three observations at a single site and from aphids and leafhoppers during one observation at a single site. For each of the five observed differences, the severity of damage in the MON 87705 plots was within the range of that for the reference soybean variety plots. Therefore, the detected differences in disease and arthropod damage ratings are unlikely to be biologically meaningful in terms of increased plant pest potential for MON 87705 compared to the conventional soybean control.

In an assessment of pest and beneficial arthropod abundance, no statistical differences were detected between MON 87705 and the conventional soybean control for 95 out of 96 comparisons (including 46 arthropod pest comparisons and 50 beneficial arthropod comparisons) among the collection intervals at the four sites (Appendix G; Tables G-8 and G-9). The single detected statistically significant difference was for bean leaf beetle in a single collection from one site, and the mean abundance value from the MON 87705 plots was within the range of that for the reference soybean variety plots. 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 87705 and arthropod pest and beneficial organisms, diseases, and abiotic stressors were not altered compared to conventional soybean. The lack of significant biological differences in plant response to abiotic stressors, disease damage, arthropod damage, and arthropod pest and beneficial insect abundance indicate that the improved fatty acid profile and glyphosate tolerance trait of MON 87705 is unlikely to be biologically meaningful in terms of increased plant pest potential.

VIII.D.3. Pollen Characteristics

APHIS considers the potential for gene flow to, and introgression of the biotechnology-derived trait into other soybean varieties and wild relatives to determine the potential for increased weedy or invasive characteristics of the receiving species. Pollen morphology and viability information are pertinent to this assessment and therefore were assessed on MON 87705. In addition, morphological characterization of pollen produced by MON 87705 and the conventional soybean control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 87705 compared to the conventional soybean.

The purpose of this evaluation was to assess the morphology and viability of pollen collected from MON 87705 compared to a conventional soybean control. Pollen was collected from MON 87705, the control (A3525), and five commercially available reference soybean varieties grown under similar agronomic conditions in a field trial in Missouri. The trial was arranged in a randomized complete block design with three replications. A minimum of 20 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 87705, the control, and the reference soybean varieties (see Appendix H).

No statistically significant differences were detected between MON 87705 and the control for percent viable pollen or pollen grain diameter (Table VIII-5). Furthermore, no visual differences in general pollen morphology were observed between MON 87705 and the control. These results demonstrate that the introduction of the modified oil profile trait did not alter the overall morphology or viability of MON 87705 pollen compared to the conventional soybean control. The lack of statistically significant differences between the pollen collected from MON 87705 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 87705 compared to the nontransformed control and other soybean varieties.

Table VIII-5. Pollen Grain Diameter and Viability Analyses

Pollen Characteristic	MON 87705* Mean (S.E.)	A3525 Mean (S.E.)	Reference Range[1] Minimum	Reference Range[1] Maximum
Viability (%)	98.3 (0.3)	96.7 (0.5)	97.2	99.6
Diameter (µm)	25.7 (0.1)	25.0 (0.5)	24.3	25.8

S.E. = Standard Error. Means based on n = 3.
* No significant differences were detected between the MON 87705 and the control ($p > 0.05$).
[1] Reference range is the minimum and maximum mean value observed among the five reference soybean varieties.

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 nitrogen-fixing plant-microbe symbiosis results in the formation of root nodules, providing an environment in which differentiated bacteria called bacteroids are capable of reducing or fixing atmospheric nitrogen. The product of nitrogen fixation, ammonia, then can be utilized by the plant. In soybean, atmospheric nitrogen is fixed into ammonia through a symbiotic association with the bacterium *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 modified crop on pest potential and the environment as well as on agricultural or cultivation practices (CFR § 340.6). Changes in the symbiotic relationship with *Rhizobiaceae* and *Bradyrhizobiaceae* could directly impact pest potential, the environment, or 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 87705 had been altered as a result of the introduction of the improved fatty acid profile and glyphosate tolerance trait 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 (Appunu and Dhar, 2006; Israel et al., 1986).

MON 87705, a conventional soybean control (A3525), and six reference soybean varieties were produced from seed planted in pots containing nitrogen-deficient potting medium 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, and 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. Detailed information on materials and methods used for symbiont evaluation is presented in Appendix I.

No significant differences were detected between MON 87705 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-6).

Based on the assessed characteristics, the results support the conclusion that the introduction of the improved fatty acid profile and glyphosate tolerance trait does not alter the symbiotic relationship between *B. japonicum* and MON 87705 compared to conventional soybean. Thus, there is no increased plant pest potential and no expected

impact to cultivation practices relative to nitrogen inputs for MON 87705 compared to the nontransformed control or other soybean varieties.

Table VIII-6. Symbiont Interaction Assessment of MON 87705 and the Control

Measurement Endpoint	Mean (S.E.)*		p-Value	Reference Range[1]	
	MON 87705	A3525		Min	Max
Nodule Number (per plant)	229 (43)	246 (39)	0.6625	140	235
Nodule Dry Wt (g/plant)	0.61 (0.08)	0.64 (0.03)	0.7160	0.52	0.73
Root Dry Wt (g/plant)	1.68 (0.11)	1.52 (0.10)	0.4228	1.54	2.22
Shoot Dry Wt (g/plant)	5.24 (0.59)	5.13 (0.38)	0.8839	5.76	7.71
Shoot Total Nitrogen (% dwt)	3.26 (0.21)	3.54 (0.12)	0.0832	2.74	3.41
Shoot Total Nitrogen (g)	0.18 (0.02)	0.18 (0.02)	0.7957	0.18	0.23

Note: Pots were arranged in eight replicated blocks (n = 8) in a greenhouse using a randomized completed block design. S.E. = standard error.
*No significant differences were detected between MON 87705 and the control ($p>0.05$).
[1]Reference range is the minimum and maximum mean value observed among six commercial reference soybean varieties.

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 improved fatty acid profile and glyphosate tolerance tolerance trait altered the plant pest potential of MON 87705 compared to the conventional soybean control A3525. Phenotypic and agronomic characteristics of MON 87705 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; observations for abiotic stressor, disease damage, arthropod damage and arthropod abundance; plant-symbiont interaction characteristics; and compositional evaluation (Section VII) of 67 different components (seven in forage, and 60 in seed).

Results from the phenotypic and agronomic assessments demonstrate that MON 87705 does not possess characteristics that would confer a plant pest risk compared to conventional soybean. Data on environmental interactions also indicate that MON 87705 does not confer any biologically meaningful increased susceptibility or tolerance to specific disease, insect, or abiotic stressors, or changes in agronomic and phenotypic characteristics. Taken together, these data support the conclusion that MON 87705 is not likely to pose increased plant pest risk compared to conventional soybean.

IX. U.S. AGRONOMIC PRACTICES

IX.A. Introduction

As part of the plant pest assessment required by 7 CFR § 340.6(c)(4) impacts to agricultural and cultivation practices must be considered. This section provides a summary of current agronomic practices in the U.S. for producing soybean and is included in this petition as a baseline to assess possible impacts to agricultural practices due to the cultivation of MON 87705. Discussions include soybean production, seed production, growth and development, general management practices (including identity preservation practices), management of weeds, insects and diseases, soybean rotational crops, and volunteer soybean management. Information presented in the previous section demonstrated that MON 87705 is no more susceptible to diseases or pests than conventional soybean. Additionally data presented in Section VIII show that, with the exception of an improved fatty acid profile and tolerance to the herbicide glyphosate, MON 87705 is phenotypically equivalent to conventional soybean. Thus, there are no changes to the inputs needed for MON 87705, and no specific impacts to most of the agronomic practices employed for production of soybean. Where there is potential for impact on agronomic practices from the deregulation of MON 87705, discussion delineating the scope and magnitude of those impacts is provided. For example, MON 87705 will be produced under an identity preservation system requiring specific management practices to preserve the value of the oil. Therefore, emphasis is placed on anticipated impacts to agronomic practices used for production of specialty type soybean uponderegulation of MON 87705. Additionally MON 87705 has a glyphosate-tolerance trait so potential impacts to crop rotation practices are considered.

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, appropriate variety selection, appropriate planting dates and plant population, and good integrated pest management practices are important for optimizing the yield potential and economic returns of soybean.

MON 87705 is expected to bring added value for growers, soybean handlers, crushers and food processors. An identity preservation system will be used for production, post-harvest handling and processing to preserve the enhanced value of MON 87705. As such, production practices, post-harvest handling of soybean, and processing will fall under a separate production system and distribution channel termed for this specialty soybean. MON 87705 is expected to utilize existing agricultural practices employed for production and identity preservation of specialty 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 a herbicide application. Soybean insects and diseases generally are

considered less problematic, although infestations can reach economic thresholds requiring treatment.

Volunteer soybean, i.e., soybean plants that have germinated and emerged unintentionally in a subsequent crop, are not considered a significant concern in rotational crops primarily because of climatic conditions and adequate control 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 87705 in the soybean production system would have a negligible impact on managing soybean volunteer plants in rotational crops such as corn, cotton, and rice, 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 plants emerge after planting, shallow cultivation will control most of the plants and effectively reduce competition with the crop. Several postemergence herbicides also are available to control volunteer soybean (conventional or glyphosate-tolerant soybean) in each of the major rotational crops.

As shown in Sections VII and VIII, with the exception of the improved fatty acid profile and glyphosate tolerance trait, no phenotypic, compositional, or environmental differences between MON 87705 and conventional soybean have been observed. Moreover herbicide-tolerant soybean is currently grown on 92% of soybean acres (USDA-NASS, 2008). Therefore, it is not anticipated that commercialization of MON 87705 in the U.S. would have a notable impact on current soybean cultivation practices, including the management of weeds, diseases, and insects.

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 $12.9 billion 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 $4.1 billion. Japan is the second largest export market with sales of $1.1 billion 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, Midwestern and portions of the Great Plains regions 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 moisture. Economic factors include expected soybean price and anticipated economic return (Boerma and Specht, 2004).

The vast majority of soybean grown in the U.S. is grown for animal feed and is usually fed as soybean meal. However, soybean is also grown as a specialty soybean product for a specific market or use.[5] Examples of specialty soybean include high protein, tofu, high oil, high oleic, non-biotechnology-derived (also referred to as non-genetically modified), and organic soybean.[6] The uses of these soybean varieties include human consumption, food processing or specialty products. The soybean varieties used in the specialty market are typically specified by buyers and end-users of soybean for production, and a premium relative to commodity soybean is paid for delivering a product that meets purity and quality standards (Pritchett et al., 2002; Elberhi, 2007; Sundstrom et al., 2002; Lee and Herbek, 2004; Muth et al, 2003; and Smyth and Phillips, 2002) for the soybean variety. Product differentiation and market segmentation in the specialty soybean industry includes mechanisms to keep track of the soybean (traceability), methods for identity preservation (IDP), including closed-loop systems, and quality assurance processes (e.g., ISO9001-2000 certification), as well as contracts between growers and buyers that specify delivery agreements. MON 87705 is an improved fatty acid profile soybean oil product and will be considered a specialty soybean product and marketed in a manner similar to other high-value specialty crops.

The U.S. soybean acreage in the past 10 years has varied from approximately 64.7 to 75.7 million acres, with the lowest acreage recorded in 2007 and the highest in 2008 (Table 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 (2009a,b), soybean was planted on approximately 75.7 million acres in the U.S. in 2008, producing 2.96 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 value of soybean reached $27.4 billion in the U.S. in 2008 (USDA-NASS, 2009a,b). In comparison, corn and wheat values in 2008 were $47.37 and $16.57 billion, respectively (USDA-NASS, 2009a,b).

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[5] http://usb.adayana.com:8080/usb/jsp/login.jsp
[6] http://usb.adayana.com:8080/usb/jsp/login.jsp

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

Managing input costs is a major component to the economics of producing a soybean crop. Key decisions on input costs include choosing what soybean varieties to plant, amounts of fertilizer to apply, and what herbicide program to use. The average operating cost for producing soybean in the U.S. in 2006 was $93.41 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 $161.43 per acre. A summary of potential production costs and returns are presented in Table IX-3.

Table IX-1. Soybean Production in the U.S., 1999 – 2008

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

Source: USDA-NASS, 2009b.

Table IX-2. U.S. Soybean Production by Region and State in 2008

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

[1] Source: USDA-NASS, 2009b.

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		**254.84**
Operating Costs:	Seed	32.30
	Fertilizer	13.05
	Chemicals	14.46
	Custom operations	6.01
	Fuel, lube and electricity	13.51
	Repairs	11.80
	Purchased irrigation water	0.11
	Interest on operating capital	2.17
Total, operating costs		**93.41**
Allocated overhead:	Hired labor	1.78
	Opportunity cost of unpaid grower's labor	15.20
	Capital recovery of machinery and equipment	60.38
	Opportunity cost of land (rental rate)	86.17
	Taxes and insurance	7.93
	General farm overhead	13.22
Total, allocated overhead		**184.68**
Total cost listed		**278.09**
Value of production less total cost listed		**(23.25)**
Value of production less operating costs		**161.43**

Supporting Information: Yield = 46 bushels/acre, Price = $5.54/bushel, Enterprise size = 268 planted acres, Irrigated = 9%, Dry land = 91%.

Source: USDA-ERS, 2006.

IX.B.2. Specialty Soybean

Commodity and specialty soybean are the two primary production and distribution systems for soybean produced in the U.S. The majority of soybean is commonly grown and marketed through commodity markets for the oil and protein content. Commodity soybean is not consumed directly, but is crushed for meal that is predominantly used for animal feed and as a minor protein source for food. The oil produced during the crushing of the soybean is used for cooking or food ingredients. The goal of the commodity supply chain is to supply a homogenous product to the enduser. The grower producing soybean for this chain has a choice from many different varieties for production; however, harvested soybean is viewed to be the same for all commodity soybean varieties. At harvest, the grower either delivers soybean to a handler or stores them on farm for later delivery. The handler is not interested in differentiating the commodity soybean for later use. Commodity soybean handlers typically have large volume storage capacity. Similarly, commodity soybean processors crush large volumes of soybean to produce homogeneous oil and meal products. The commodity system is designed to maximize efficiency at a low profit margin resulting in comingling of different sources of soybean that does not affect the price received for the final product. This production system has been in place in the U.S. since the production of soybean began in earnest in the 1960s (Sonka et al., 2004).

In recent years, there has been an increased demand by consumers and food processers for soybean that has specific physical or chemical characteristics that are required by certain customers to meet specific food or feed needs. As a result a separate specialty soybean channel has developed. This production system and distribution channel is focused on value-added traits that involve much smaller volumes than commodity soybean (Sonka et al., 2004). Specialty soybean varieties are produced on approximately 12% of the U.S. soybean acreage[7] (and according to the Midwest Shippers Association (MSA, 2009), this acreage could grow to over 25% of the crop acreage in certain states within the next decade. This supply chain typically consists of a specialty firm that contracts production of a specific variety and sets standards for quality of the harvested soybean (Lee and Herbek, 2004). In return, growers receive a premium over the price paid for commodity soybean. Growers may store harvested soybean on farm or deliver the product directly to a processor or to special containers for international shipment. The goal of this identity preserved system is to minimize handling so that value is maintained. The cost incurred from an identify preservation system is offset by the higher value received for the final product.

According to the American Soybean Association (2009), specialty soybean can be grouped into ten broad categories: non-biotechnology-derived, certified seed, organic food-grade, low saturated fat, clear hilum, tofu, natto, high sucrose, high oleic, low

[7] http://usb.adayana.com:8080/usb/jsp/login.jsp: Percent U.S. soybean acreage estimate based on U.S. Domestic Consumption by Segment – 2008/09. D. Ludwig, personal communication, 2009.

linolenic and high protein. The categories refer to soybean with characteristics such as altered seed composition (e.g., low saturated fat, high sucrose, high oleic, low linolenic, and high protein), varieties of soybean with unique physical characteristics suited to their specific uses (e,g., clear hilum for direct human consumption), or refer to a production process (e.g., organic, certified seed). The categories are not meant to be exclusive; for example, soybean used to produce natto or tofu may employ organic production processes, and soybeans from all of the categories are often derived from varieties produced according to certified seed production practices. Tofu and soymilk produced from the tofu soybean category represent a large segment of the specialty soybean market and are produced from unique soybean varieties that have clear hilum and large seed size (Lee and Herbek, 2004). Tofu varieties also must be high in protein (40% or higher) and low in oil concentration compared to commodity soybean. Clear hilum and other characteristic are required for soybean used in the production of other soybean food products consumed directly by humans such as natto, soybean sprouts, edamame (vegetable soybean), and soy nuts (Lee and Herbek, 2004; UK, 2009). Organic food-grade and non biotechnology-derived soybean varieties are identity protected and produced not to contain biotechnology-derived traits. Organic soybean have additional production restrictions requiring the soybean to be produced using no synthetic fertilizers or pesticides. In recent years, public and private soybean breeders have developed soybean varieties with improved nutritional characteristics (e.g., high sucrose, high oleic, low linolenic and high protein) and MON 87705 is considered part of this trend towards production of value-added soybean products. The characteristics or modifications in this group include reducing the need for hydrogenation of the soybean oil, increasing sugar concentration, increasing protein concentration, decreasing concentration of saturated and polyunsaturated fats, and lipoxygenase free soybean which removes some of the flavors that are objectionable to some consumers (Lee and Herbek, 2004).

The majority of specialty soybean varieties are offered in Maturity Group II and early Group III varieties which are adapted to the upper Midwest/Great Plains region (Section IX.C) (Lee and Herbek, 2004). Maturity Group II and III soybean varieties are grown on approximately 42-45 million acres, occupying the largest percentage of soybean acreage (see Section IX.C). The varieties were developed for this area primarily due to proximity to processing facilities as well as international routes of shipment and to take advantage of efficiencies in soybean breeding programs (S. Joehl, personal communication, April 2009). With a few exceptions, the agronomic or management practices for growing specialty soybean from planting to harvest are similar to commodity soybean (Lee and Herbek, 2004). Because special varieties are used in the production of each specialty soybean, the variety selection is dictated by the specialty soybean buyer or processor.

Non biotechnology-derived and organic soybean by definition must be produced utilizing only conventional soybean varieties. Weed control is extremely important for specialty soybean to maintain a high yield potential and because weeds, such as nightshade (*Solanum nigrum*), can stain harvested soybean, which is particularly undesirable in food-grade soybean (TCM, 2008). Because organic soybean must be grown without synthetic fertilizers or pesticides for three or more years prior to the current crop of soybean, fertilization and pest management is much more difficult (Lee and Herbek, 2004). Weed control in organic soybean relies on a combination of crop rotation, tillage, in-crop

cultivation, and hand-weeding. Insect and disease control is managed primarily through crop rotation. Certain approved pesticides are permitted in this specialty soybean production.

All equipment and storage facilities for specialty soybean must be clean of seed from other soybean varieties or plants, dirt, pathogens and other foreign material. Some soybean contracts may require a special inspection of the handling and storage facilities. The specialty soybean for soybean foods may require special harvesting equipment since some of these soybeans are harvested before full maturity (e.g., edemame or vegetable soybean).

IX.B.3. Identity Preservation

Identity Preservation (IDP) refers to a system of production, handling, and marketing practices that maintains the integrity and purity of agricultural commodities (Sundstrom et al., 2002). Commodity grains, on the other hand, are marketed in mass according to USDA grading standards. Specialty crops require some form of segregation or full-scale identity preservation to keep these grains separate from commodity grains (Elberhi, 2007). This market segmentation within the grain channeling system is driven by the need to preserve market value or ensure a specified purity of the product. With certain specialty crops, IDP is required to prevent accidental or unintended commingling (e.g., non biotechnology, organic) or to segregate products that are approved only for certain uses (industrial use only).

IDP grain production has been in existence for a long time. Agricultural producers have over many decades developed practices that allowed for differentiation between food vs. feed grain, or grain vs. seed production, or organic vs. non organic (Massey, 2002). Seed certification programs such as those used by the Association of Official Seed Certifying Agencies are often cited as the model of IDP systems. These programs date back to the 1920s and 1930s when the certification process was implemented to verify the genetic purity of seed made available to growers (see Section IX.B.4). Standards were established to ensure production of seed from known pedigree with high purity and quality. Similarly, commodity grain traders, marketing organizations, and food processors have established purity and quality tolerances for specific end-product uses. The need for segregation and IDP production systems has increased with the development of specialty crops or crops with special output traits, such as high oil corn, high oleic sunflower, and low-linolenic soybean (Sundstrom et al., 2002).

The production of IDP grains requires special processes in order for growers to meet buyers' criteria for variety identification, composition, and quality. Buyers of IDP soybean typically contract for the production of seed-variety specific soybean and work directly with seed suppliers, growers, independent certification agencies, intermediate processors, and freight companies to deliver the preferred product within specified tolerances.[8] Contract specifications are written to ensure the delivery of the desired

[8] United Soybean Board, International Buyer's Guide at http://www.ussoyexports.org/ussoy/buyersguide/Chap6.pdf

attribute-specific product and that predetermined management practices are used (Massey, 2002). Lack of compliance with a product specification can lead either to a price discount or rejection of a shipment by the buyer. Depending on the end use, contracts can be extensive, defining many of the production, harvest and storage activities, or less stringent dealing more with pricing, quality specifications and only the most critical production practices. Premiums are paid to growers for the additional risk and management only when the grain meets the contract specifications. Premium prices provide the impetus to maintain the specialty grain's purity and identity separate from commodity grains (Massey, 2002). Premiums are affected by various factors, including the proximity of suppliers to buyers and the cost and availability of substitutes (Sundstrom et al., 2002). For many trait-specific crops, price premiums rise or fall depending on the supply conditions for the generic commodity.

Growers of IDP grains must have good managerial ability and implement certain management practices to produce and deliver grain possessing the desired physical and chemical characteristics. The production and marketing of trait-specific grains involves additional financial risks (Elberhi, 2007). The growers' managerial ability can affect both yield performance and proficiency in meeting the contract specifications. From a soybean buyer's perspective, contracts help the buyer meet the demand for specific product qualities, improve cost efficiencies of product processing, and reduce transaction costs.

While specific IDP production practices vary depending on the characteristics of the product to be delivered, general elements are implemented to ensure that the end-user or processor receives the grain or end-product with the intended identity and desired quality. As mentioned above, many IDP systems were developed using the principles similar to those used in seed certification. IDP production begins with a system of standards, records, and auditing that are put in place throughout the entire crop production, harvesting, handling, and marketing process (Sundstrom et al., 2002). Some key considerations in the establishment of an IDP system include: 1) planted seed identity and tolerances, 2) appropriate field isolation, 3) inspection and clean-out of equipment and facilities, 4) end-product sampling and testing, and 5) record maintenance and identity labeling (Sundstrom et al., 2002). Each of these components are described in greater detailed below:

Planting Seed Identity and Tolerances

The purity and identity of starting seed should be tested and confirmed. The purity of the seed stock should equal or exceed the purity standards of the desired final product. Single or multiple quality tolerances may be established in specialized IDP programs based on market-driven standards.

Field Isolation

Crops must be isolated either spatially or temporally from pollen sources that could impact the quality or purity of the harvested seed. The amount of isolation depends on flower characteristics, sexual compatibility with neighboring crops, pollen quantity and viability, and mode of pollen dissemination. A self-pollinating crop, such as soybean, requires relatively small isolation distances to effectively preclude cross-pollination.

Equipment and Facilities

Equipment used in production should be cleaned and inspected before and after use for IDP crop. Storage facilities and transporting vehicles are cleaned and inspected to assure that segregation is maintained and no physical contamination occurs.

Sampling and Testing

In some cases, the IDP grain is sampled and tested at various stages to confirm the product identification, purity, and quality. Special consideration should be given to sampling and testing techniques that ensure reliable results.

Record Maintenance and Identity Labeling

Records typically are maintained on field designations, harvest amounts, storage bin locations, and product transfers. IDP products must be identified, segregated, and labeled in the market chain.

Because value capture is also a vital part of IDP productions systems, growers must assure markets or buyers for these IDP crops are available, especially if the crop is not being grown under contract. The soybean industry has collaborated to foster the development and availability of several specialty soybean and soybean oil markets.[9] As an example, the soybean industry initiated a program called the Low-Linolenic Locater, an internet-based program to assist growers in locating the closest processor or elevator that contracts acreage and offers premiums for low-linolenic soybean.

As with current IDP systems used for specialty soybean to preserve market value or ensure purity of the product, MON 87705 will employ an IDP system based on established practices. The IDP practices will be implemented to ensure that the end-user or processor receives the soybean with the identity, fatty acid composition of the oil, and desired quality for this value-added specialty soybean.

IX.B.4. Soybean Seed Production

Standardized seed production practices are responsible for maintaining high-quality seed stocks, an essential basis for U.S. agriculture. By the early 20th century, agronomists learned how to develop specific plant varieties with desirable traits. In the U.S., state agricultural experiment stations developed many seed varieties that were distributed to growers for use. Seed was saved by growers and later sold to neighbors; however, the desirable traits of the varieties often were lost through random genetic changes and contamination with other crop and weed seed (Sundstrom et al., 2002). The value of seed quality (including genetic purity, vigor, and presence of weed seed, seed-borne diseases, and inert materials, such as dirt) was quickly identified as a major factor 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

[9] Qualisoy: Low-linoleic locator at http://www.qualisoy.com\

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 represents state and private seed certification in the U.S., and includes international member countries in North and South America, Australia, and New Zealand.

Soybean seed is separated into four seed classes: 1) breeder, 2) foundation, 3) registered, and 4) certified (AOSCA, 2009a). 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 produced predominately to meet or exceed certified seed standards. This section of the petition will provide a broad overview of the practices used in producing certified seed.

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

The U.S. soybean production for all purposes has varied from approximately 64.7 to 75.7 million acres in the past ten years, with the lowest acreage recorded in 2007 and the highest in 2008 (USDA-NASS, 2009 b; Table IX-1). This range of soybean acreage would require between 105 - 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 that produce and sell seed, such as Monsanto Company, Pioneer Hi-Bred International, Syngenta Seeds, Kruger Seed Co., Becks Hybrids, and tollers, which are companies that produce but do not sell certified seed, such as 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 International Organization for Standardization (ISO) certified and; therefore, includes internal and external audits (ISO, 2009). ISO standards ensure desirable characteristics of seeds and services, such as quality, safety, reliability, and efficiency. The ISO standards represent an international consensus on good management practices with the aim of ensuring that the organization can consistently deliver excellent product or services. The standards not only must meet the customer's requirements and applicable seed regulatory requirements, but must aim to enhance customer satisfaction and achieve continual improvement of its performance in pursuit of these objectives.

The field operations and management practices for producing soybean seed are similar to normal soybean production. However, special attention is needed in certain areas to produce seed with high quality, high germination rates, and high genetic purity (Helsel and Minor, 1993). General guidelines specific for seed production are discussed below. The seed production field should not have been planted with soybean the previous crop in order to avoid volunteer soybean plants (even though the risk of soybean volunteer plants is negligible) and to 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 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 requirement of five feet (AOSCA, 2009a). 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 to ensure proper bin cleanout. Seed is inspected for appearance, disease, discoloration, seed coat, mechanical damage, inert matter, and weed seed. Warm and cold germination tests are conducted on all seed lots to verify acceptable germination rates. Many seed companies will also conduct tetrazolium staining tests to assess seed viability.

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

MON 87705 seed will be produced in the same manner as commercially certified soybean seed, such that it will meet all state and federal seed standards and labeling requirements.

IX.C. Production Management Considerations

Pre-Season

Crop rotation, tillage system, row spacing, planting equipment, seed or variety selection(s), and soil fertility require production decisions well in advance of planting the soybean crop. Many of the decisions in this area are made prior to or immediately after harvest of the previous crop. There are many benefits to crop rotation, with the majority of the soybean acreage planted in a two-year corn-soybean rotation (see Section 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 usually will be a long-term commitment, provided the system is successful. A decision to change row spacing is a similar long-term commitment that generally requires new equipment.

The benefits of conservation tillage or no-till systems are well documented and include reduced soil erosion, reduced fuel and labor costs, and conservation of soil moisture. 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 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 and would not normally be found in these 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.

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

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

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

Soybean variety selection is crucial for high yield and quality, and is the foundation of an effective management plan (Pedersen, 2008a). 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 20 years consistently shows that row spacing of less than 20 inches is preferred for soybean regardless of tillage system, rotation sequence or planting date (Pedersen, 2008a). In the southern states, the advantage from narrow rows is less consistent and less beneficial. In 2000, approximately 40% of soybean was planted in row spacing of 10 inches or less, 27% in 10.1 to 28.5 inches, and 33% in rows wider than 28.5 (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 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 77 ºF (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 50 ºF 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 of the state and the first week of May in the northern one-third of the state (Pedersen, 2008a). In the Southern U.S., planting adapted varieties before late April results in shorter plants and, in many cases, lower yields than when the same varieties are planted in May or early June. Planting after early June generally decreases plant height and yield due to water shortages in July and August.

Variations in plant spacing through row spacing and plant population have a significant effect on canopy development and soybean yield. Soybean has the ability to produce good yield over a wide range of plant populations. Most soybean varieties have the ability to branch and adjust the number of pods on branches to compensate for large differences in seeding rate. Maximum yields generally require planting rates that result in about 2.5 to 5 plants per square foot (Hoeft et al., 2000). Therefore, a full stand of soybean is approximately eight to ten plants per foot of row at harvest for 40-inch rows, six to eight plants per foot of row in 30-inch rows, four to six plants in 20-inch rows, and two to three plants in 10-inch rows. This translates to 109,000 to 218,000 plants per acre at harvest. Higher populations are recommended in narrow rows for maximum yields because plants are more uniformly spaced in narrow rows. Seeding rates are generally 10 to 25% higher than the desired harvest population, especially in no-till, to account for the losses in germination, emergence, and seedling diseases. The accuracy of the planting equipment also can impact the decision on seeding rate. Soybean seed traditionally has been sold by weight. Therefore, the 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, or mefenoxam) to prevent damping-off diseases may be beneficial when planting in cold, wet soils, using reduced till and no-till planting systems, and when planting seed with a low germination rate (<80%) or low seed vigor.

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

Mid to Late Season

Ideal daytime temperatures for soybean growth are between 75 ºF and 85 ºF (Hoeft et al., 2000). Warmer temperatures result in larger plants and earlier flowering. Sustained temperatures below 75 ºF 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 70 ºF. 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 generally are grown in the southern U.S. Indeterminate and determinate varieties also differ in flowering characteristics. Indeterminate plants generally bloom first at the fourth or fifth node and progress upward. Flowering on determinate plants begins at the eight or tenth node and progresses both downward and upward.

The first appearance of flowers signals the beginning of the reproductive stage, namely the R1 stage (Hoeft et al., 2000). The reproductive period consists of flowering, pod set, and seed formation. Climatic conditions such as temperature and moisture supply during the flowering period will affect the number of flowers. The soybean plant does not form a pod 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 because these conditions promote the development of reproductive-stage diseases.

Harvest Season

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

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

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 also can interfere with harvest, causing extra wear on harvest equipment (Pedersen, 2008a). The primary factors affecting soybean yield loss from weed competition are the weed species, weed density, and the duration of the competition. When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75% (Dalley et al., 2001). Generally, the competition 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. The most common weeds in soybean for each of the three major growing regions are presented in Tables IX-4, IX-5 and IX-6.

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).

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

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

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

Sources:

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

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

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

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

KY, MO: Webster et al., 2005.

MI: Davis, et al., 2005. List is not ranked in order of importance or frequency.

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

ND: Zollinger, 2000.

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

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

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

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

Sources:

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

AR: Ken Smith, Extension Weed Specialist, University of Arkansas - Personal communication (2006).

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

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

[1] Number provided in parenthesis is the number of states out of the eight total states in the Eastern Coastal region reporting each weed as a common weed. Data were not available for DE in soybean.

Sources:

DE, MD, NJ, PA: WSSA, 1992.

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

NY: Russell Hahn, Extension Weed Specialist, Cornell University – Personal Communication (2006).

VA: Scott Hagood, Extension Weed Specialist, Virginia Tech – Personal Communication (2006).

Cultural and mechanical weed control practices are important components of an effective weed management program (Baumann et al., 2008). Crop rotation, narrow row spacing and planting date are a few of the crop management practices that are implemented to provide the crop with a competitive edge over weeds. Although the primary purpose of tillage is for seedbed preparation, tillage is still used to supplement weed control with selective herbicides in soybean production. Approximately 98% of the soybean acreage received an herbicide application in 2006 indicating the importance of excellent weed control in maximizing soybean yield (USDA-ERS, 2007). 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, i.e., planting Roundup Ready soybean and applying glyphosate in crop, has become the standard weed control program in U.S. soybean production and is planted on 92% of the soybean acreage (USDA-NASS, 2008).

Herbicides provide effective and economical control of weeds in soybean. The risk of weeds developing resistance to herbicides and the potential impact of resistance on the usefulness of a herbicide vary greatly across different mechanisms of action and are dependent on a combination of factors, such as selection pressure, herbicide soil residual activity, herbicide chemistry, prolific seed production and high genetic variation in plants. Weed-resistance management programs that integrate the use of herbicides with different mechanisms of action and short residual activity times in soil reduce selection pressure (Prather et al., 2000). In conjunction with crop rotation, which may allow the grower to manipulate planting times to avoid early-season weed germination (Jordan et al., 1995) and to use mechanical as well as chemical weed control methods, these practices impede the development of herbicide resistance in weeds. Monsanto invests

considerably in research to understand the proper uses and stewardship of the glyphosate molecule. This research includes an evaluation of some of the factors that can contribute to the development of weed resistance. Detailed information regarding glyphosate weed resistance management is presented in Appendix L.

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 (USDA-NASS, 2007).

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

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 also can 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 $1.5 billion in the U.S. (Pedersen, 2008a). SCN can cause yield losses up to 50%, where this pest in 2004 alone caused an estimated loss of 50 million bushels 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 also is 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 demonstrate resistance to specific taxonomic races of 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. At this time, foliar application of fungicides is the standard disease-management practice to limit yield losses due to soybean rust.

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

IX.G. Crop Rotation Practices in Soybean

The well-established farming practice of crop rotation is still a key management tool for growers. The purposes of growing soybean in rotation are to improve yield and profitability of one or both crops over time, decrease the need for nitrogen fertilizer on the crop following soybean, increase residue cover, mitigate or break disease, insect, and weed cycles, reduce soil erosion, increase soil organic matter, improve soil tilth and soil physical properties, and reduce runoff of nutrients, herbicides, and insecticides (Boerma and Specht, 2004; Al-Kaisi et al., 2003). According to USDA Economic Research Service, 95% of the soybean-planted acreage has been in some form of a crop rotation system since 1991 (USDA-ERS, 2001). Corn- and wheat-planted acreage has been rotated at a slightly lower level of 75% and 70%, respectively. Although the benefits of crop rotations can be substantial, the grower must make cropping decisions by evaluating both the agronomic and economic returns 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 (Hoeft et al., 2000; Al-Kaisi et al., 2003). Corn and soybean occupy more than 80% of the farmland in many of the Midwestern states, and the two-year cropping sequence of soybean-corn is used most extensively in this region. However, a soybean crop sometimes is grown after soybean and then rotated to corn in a 3-year rotation sequence (soybean-soybean-corn) in the Midwest. Compared to corn, soybean shows a greater yield response to being grown after a number of years without soybean. The yield of both corn and soybean is approximately 10% higher when grown in rotation than when either crop is grown continuously (Hoeft et al., 2000).

A combination of conservation tillage practices and crop rotation has been shown to be very effective in improving soil physical properties. Long-term studies in the Midwest indicate that the corn-soybean rotation improves yield potential of no-till systems compared to continuous corn production (Al-Kaisi, 2001). The reduction in yield of

continuous corn production in no-till systems is attributed to low soil temperature during seed germination, which is evident on poorly drained soils under no-till practices.

Crop rotations may change over a long period of time due to economic conditions and market opportunities. Roundup Ready soybean has provided growers more profit opportunities than conventional soybean primarily by reducing input costs (Gianessi, 2005). In addition, Roundup Ready soybean has provided growers greater flexibility to grow soybean in fields with weed infestations, which previously were considered to be too problematic or unproductive for growing.

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

Agronomic practices such as rotations for soybean vary from state to state. However, there are similarities among states within certain growing regions. This section provides a detailed description and quantitative assessment of the rotational cropping practices immediately following soybean production, by state. This assessment accounts for about 99% of the total soybean acreage. These data are presented in Tables IX-7 through IX-10.

The majority of the U.S. soybean acreage (68.6%) is rotated to corn (Table IX-7). The second largest rotational crop following soybean is soybean. Approximately 14.5% of the soybean acreage is rotated back to soybean the following year. Wheat follows soybean on approximately 11.2% of the U.S. soybean acreage, with cotton, rice, and sorghum the next largest rotational crops following soybean. However, these three crops were planted on only 4.6% of the soybean acreage. Other minor rotational crops that follow soybean production are listed in Tables IX-7 through IX-10.

Column J of each table provides the percentage of soybean acreage as a function of the total rotational crop acreage to indicate the level that soybean is the primary crop preceding the rotational crops. For the entire country (Table IX-7), this percentage is 35.3% indicating that soybean is a major crop preceding these rotational crops. The percentage of soybean as a preceding crop varies widely in different states, which ranges from 16.8% (KS) to 95.2% (NJ). In the Midwest region where 82% of the soybean is grown, 34.6% of the rotational crop area was planted with soybean during the previous growing season.

One rotation choice available to growers is to plant another Roundup Ready crop following the production of glyphosate-tolerant soybean. To determine the likelihood that the rotational crops planted after MON 87705 will be another Roundup Ready crop, an assessment also has been provided in Tables IX-7 through IX-10. This assessment is based on current agronomic practices following soybean production. Roundup Ready

alfalfa, canola, corn, cotton, soybean, and sugar beets have been nonregulated by the USDA and were considered as potential Roundup Ready crops following soybean production. For the purposes of this assessment, the adoption rates used for Roundup Ready corn, cotton, and soybean in 2008 were obtained from the USDA-NASS Acreage Summary report (2008c). The percentages for Roundup Ready corn, cotton, and soybean in the following tables were assumed to be the total percentage of herbicide-tolerant crops because the USDA-NASS report does not show the percentages of each individual herbicide-tolerant trait. Therefore, this is a slight overstatement for Roundup Ready corn, cotton, and soybean since other herbicide-tolerant traits are planted. Roundup Ready sugar beets were commercially introduced in 2008, although the USDA-NASS report does not provide planting information for this biotechnology-derived crop. Therefore, a 90% adoption rate will be assumed for future plantings. Considering Roundup Ready alfalfa was nonregulated by the USDA, but is currently not available in the marketplace, a future market adoption estimate will be used. An adoption rate of 50% will be assumed for Roundup Ready alfalfa.

This assessment showed that the percentage of the total rotational crop acreage that may be rotated from Roundup Ready soybean to another Roundup Ready crop (Table IX-7 - Column K) is estimated to be 21.0% in the U.S. and ranges from 7.1% (KS) to 78.3% (MS) across the soybean growing states. The percentage is 20.0% in the Midwest region, which is the largest soybean growing region.

Table IX-7. Rotational Practices in the U.S. Following Soybean Production

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in the U.S.[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
United States	**75037**	Corn	80130	64.3	51500	68.6	64.5	33214		
		Soybean	75037	14.5	10866	14.5	92.6	10062		
		Sorghum	4020	20.9	841	1.1	NA			
		Cotton	3767	41.7	1570	2.1	79.3	1245		
		Wheat	37414	22.4	8396	11.2	NA			
		Barley	2159	1.9	41	0.05	NA			
		Oats	1995	4.9	98	0.1	NA			
		Rice	2301	45.3	1042	1.4	NA			
		Alfalfa	1864	8.7	162	0.2	50	81		
		Sugar Beets	830	17.3	144	0.19	90	129		
		Potatoes	334	9.6	32	0.04	NA			
		Dry Beans	1183	3.0	35	0.05	NA			
		Dry Peas	520	7.3	38	0.05	NA			
		Millet	250	16.4	41	0.05	NA			
		Flax	345	22.0	76	0.10	NA			
		Other[9]	452	34.3	155	0.2	NA			
			Total: 212601		**Total: 75037**			**Total: 44731**	**35.3**	**21.0**

The U.S. summary (Table IX-7) was developed by compiling the data from all three regional summaries. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2008 planting data (USDA-NASS, 2008); "other" crop and newly seeded alfalfa acreages are based on 2008 planting data from the Individual States data which was obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp.

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

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

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

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

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

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

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

[9] Various vegetables.

Table IX-8. Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
Region	**62150**	Corn	72260	65.7	47480	76.4	65.4	31040		
		Soybean	62150	7.9	4885	7.9	92.6	4523		
		Sorghum	3553	18.8	670	1.1	NA			
		Cotton	341	22.9	78	0.1	87.2	68		
		Wheat	32039	25.3	8102	13.0	NA			
		Barley	1929	2.1	41	0.07	NA			
		Oats	1590	6.2	98	0.2	NA			
		Rice	200	91.0	182	0.3	NA			
		Alfalfa[9]	1617	10.0	162	0.3	50	81		
		Sugar Beets	830	17.3	144	0.2	90	129		
		Potatoes	278	11.6	32	0.05	NA			
		Dry Beans	1166	3.0	35	0.06	NA			
		Dry Peas	520	7.3	38	0.06	NA			
		Millet	250	16.4	41	0.07	NA			
		Flax	345	22.0	76	0.1	NA			
		Other[10]	342	25.3	87	0.1	NA			
			Total: 179410		**Total: 62150**			**Total: 35841**	**34.6**	**20.0**
IL	**9200**	Corn	12100	71	8556	93.0	67%	5733		
		Soybean	9200	3	230	2.5	87%	200		
		Sorghum	80	92	74	0.8	NA			
		Wheat	1200	28	340	3.7	NA			
			Total: 22580		**Total: 9200**			**Total: 5933**	**40.7**	**26.3**
IN	**5450**	Corn	5700	86	4905	90	71%	3483		
		Soybean	5450	5	273	5	96%	262		
		Wheat	580	47	273	5	NA			
			Total: 11730		**Total: 5450**			**Total: 3744**	**46.5**	**31.9**

Table IX-8 (cont.). Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
IA	**9750**	Corn	13300	71	9458	97	68	6431		
		Soybean	9750	2	195	2	95	185		
		Alfalfa[9]	125	78	98	1	50	49		
			Total: 23175		**Total: 9750**			**Total: 6665**	**42.1**	**28.8**
KS	**3300**	Corn	3850	43	1650	50	65	1073		
		Soybean	3300	10	330	10	95	314		
		Sorghum	2900	6	165	5	NA			
		Wheat	9600	12	1155	35	NA			
			Total: 19650		**Total: 3300**			**Total: 1386**	**16.8**	**7.1**
KY	**1390**	Corn	1210	98	1182	85	54	638		
		Soybean	1390	10	139	10	87	121		
		Wheat	580	12	70	5	NA			
			Total: 3180		**Total: 1390**			**Total: 759**	**43.7**	**23.9**
MI	**1900**	Corn	2400	55	1330	70	57	758		
		Soybean	1900	5	95	5	84	80		
		Wheat	730	65	475	25	NA			
			Total: 5030		**Total: 1900**			**Total: 838**	**37.8**	**16.7**
MN	**7050**	Corn	7700	70	5358	76	69	3697		
		Soybean	7050	3	212	3	91	192		
		Wheat	1925	66	1269	18	NA			
		Sugar beets	440	24	106	1.5	90	95		
		Dry Beans	150	24	35	0.5	NA			
		Other[11]	203	35	71	1	NA			
			Total: 17468		**Total: 7050**			**Total: 3984**	**40.4**	**22.8**

Table IX-8 (cont.). Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
MO	**5200**	Corn	2800	98	2756	53	43	1185		
		Soybean	5200	30	1560	30	92	1435		
		Sorghum	90	116	104	2	NA			
		Cotton	306	25	78	1.5	87	68		
		Wheat	1250	42	520	10	NA			
		Rice	200	91	182	3.5	NA			
			Total: 9846		**Total: 5200**			**Total: 2688**	**52.8**	**27.3**
NE	**4900**	Corn	8800	42	3675	75	59	2168		
		Soybean	4900	10	490	10	97	475		
		Sorghum	300	82	245	5	NA			
		Wheat	1750	28	490	10	NA			
			Total: 15750		**Total: 4900**			**Total: 2644**	**31.1**	**16.8**
ND	**3800**	Corn	2550	45	1140	30	65	741		
		Soybean	3800	21	798	21	94	750		
		Wheat	9230	19	1710	45	NA			
		Sugar Beets	208	18	38	1	90	34		
		Dry Peas	520	7	38	1	NA			
		Flax	335	23	76	2	NA			
			Total: 16643		**Total: 3800**			**Total: 1525**	**22.8**	**9.2**
OH	**4500**	Corn	3300	95	3150	70	54	1701		
		Soybean	4500	10	450	10	89	401		
		Wheat	1120	80	900	20	NA			
			Total: 8920		**Total: 4500**			**Total: 2102**	**50.4**	**23.6**

Table IX-8 (cont.). Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
SD	**4100**	Corn	4750	62	2952	72	88	2598		
		Soybean	4100	2	82	2	97	80		
		Sorghum	170	48	82	2	NA			
		Wheat	3661	22	820	20	NA			
		Barley	63	65	41	1	NA			
		Oats	220	37	82	2	NA			
		Millet	110	37	41	1	NA			
			Total: 13074		**Total: 4100**			**Total: 2677**	**31.4**	**20.5**
WI	**1610**	Corn	3800	36	1369	85	61	835		
		Soybean	1610	2	32	2	90	29		
		Wheat	373	22	81	5	NA			
		Oats	270	6	16	1	NA			
		Alfalfa[9]	420	15	64	4	50	32		
		Potatoes	63.5	51	32	2	NA			
		Other[12]	139.2	12	16	1	NA			
			Total: 6676		**Total: 1610**			**Total: 896**	**24.1**	**13.4**

The Midwest region summary (Table IX-8) was developed by compiling the data from all the states within the region. Unlike the individual state data, the data in Column G for this regional summary were obtained by dividing Column F by Column B and the data in Column H were obtained by dividing Column I by Column F. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2008 planting data (USDA-NASS, 2008); "other" crop and newly seeded alfalfa acreages are based on 2008 planting data from the Individual States data which were obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp.

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

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

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

[5] Roundup Ready rotational crop adoption rates for corn, soybean and cotton are based on 2008 planting data (USDA-NASS, 2009a). The percentages for Roundup Ready corn, cotton and soybean represent the percentages for total herbicide-tolerant traits. Percentages of herbicide-tolerant alfalfa and sugar beets are future market adoption estimates.

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

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

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

[9] Newly seeded alfalfa.

[10] Various vegetables.

[11] Sweet corn and green peas.

[12] Sweet corn, green peas and onions.

Table IX-9. Rotational Practices Following Soybean Production in the Mid-South Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
Region	**8200**	Corn	2630	43.1	1132	13.8	54.0	612		
		Soybean	8200	63.0	5164	63.0	93.3	4820		
		Sorghum	368	46.5	171	2.1	NA			
		Cotton	1860	32.6	606	7.4	82.8	502		
		Wheat	2850	9.4	267	3.3	NA			
		Rice	2101	40.9	860	10.5	NA			
			Total: 18009		**Total: 8200**			**Total: 5934**	**45.5**	**32.9**
AL	**360**	Corn	260	48	126	35	54	68		
		Soybean	360	5	18	5	87	16		
		Cotton	290	62	180	50	80	144		
		Wheat	240	15	36	10	NA			
			Total: 1150		**Total: 360**			**Total: 228**	**31.3**	**19.8**
AR	**3300**	Corn	440	53	231	7	54	125		
		Soybean	3300	64	2112	64	94	1985		
		Sorghum	125	53	66	2	NA			
		Wheat	1070	22	231	7	NA			
		Rice	1401	47	660	20	NA			
			Total: 6336		**Total: 3300**			**Total: 2110**	**52.1**	**33.3**
LA	**1050**	Corn	520	20	105	10	54	57		
		Soybean	1050	65	683	65	87	594		
		Sorghum	120	88	105	10	NA			
		Cotton	300	53	158	15	79	124		
			Total: 1990		**Total: 1050**			**Total: 775**	**52.8**	**38.9**

Table IX-9 (continued). Rotational Practices Following Soybean Production in the Mid-South Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
MS	**2000**	Soybean	2000	90	1800	90	97	1746		
		Rice	230	87	200	10	NA			
			Total: 2230		**Total: 2000**			**Total: 1746**	**89.7**	**78.3**
TN	**1490**	Corn	690	97	671	45	54	362		
		Soybean	1490	37	551	37	87	480		
		Cotton	285	94	268	18	87	233		
			Total: 2465		**Total: 1490**			**Total: 1075**	**60.4**	**43.6**

The Mid-South region summary (Table IX-9) was developed by compiling the data from all the states within the region. Unlike the individual state data, the data in Column G for this regional summary were obtained by dividing Column F by Column B and the data in Column H were obtained by dividing Column I by Column F. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2008 planting data (USDA-NASS, 2008); "other" crop and newly seeded alfalfa acreages are based on 2008 planting data from the Individual States data which were obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp.

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

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

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

[5] Roundup Ready rotational crop adoption rates for corn, soybean and cotton are based on 2008 planting data (USDA-NASS, 2009a). The percentages for Roundup Ready corn, cotton and soybean represent the percentages for total herbicide-tolerant traits. Percentages of herbicide-tolerant alfalfa and sugar beets are future market adoption estimates.

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

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

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

Table IX-10. Rotational Practices Following Soybean Production in the Eastern Coastal Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
Region	**4687**	Corn	5240	55.2	2892	61.7	54.0	1562		
		Soybean	4687	17.6	827	17.6	87.0	719		
		Cotton	1566	56.6	887	18.9	76.1	675		
		Wheat	2525	1.1	27	0.6	NA			
		Other[9]	110	49.5	54	1.2	NA			
			Total: 14128		**Total: 4687**			**Total: 2956**	**33.2**	**20.9**
DE	**195**	Corn	160	98	156	80	54	84		
		Soybean	195	20	39	20	87	34		
			Total: 355		**Total: 195**			**Total: 115**	**54.9**	**33.3**
GA	**430**	Corn	370	12	43	10	54	23		
		Cotton	940	41	387	90	78	302		
			Total: 1310		**Total: 430**			**Total: 325**	**32.8**	**24.8**
MD	**495**	Corn	460	97	446	90	54	241		
		Soybean	495	10	50	10	87	43		
			Total: 955		**Total: 495**			**Total: 284**	**51.8**	**29.7**
NJ	**92**	Corn	85	97	83	90	54	45		
		Other[10]	11.6	79	9	10	NA			
			Total: 97		**Total: 92**			**Total: 45**	**95.2**	**46.3**
NY	**230**	Corn	1090	20	219	95	54	118		
		Other[11]	33.5	34	12	5	NA			
			Total: 1124		**Total: 230**			**Total: 118**	**20.5**	**10.5**
NC	**1690**	Corn	900	90	811	48	54	438		
		Soybean	1690	25	423	25	87	368		
		Cotton	430	98	423	25	76	321		
		Other[12]	65	52	34	2	NA			
			Total: 3085		**Total: 1690**			**Total: 1127**	**54.8**	**36.5**

Table IX-10 (continued). Rotational Practices Following Soybean Production in the Eastern Coastal Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
PA	**435**	Corn	1350	32	426	98	54	230		
		Soybean	435	2	9	2	87	8		
			Total: 1785		**Total: 435**			**Total: 238**	**24.4**	**13.3**
SC	**540**	Corn	355	84	297	55	54	160		
		Soybean	540	30	162	30	87	141		
		Cotton	135	40	54	10	68	37		
		Wheat	220	12	27	5	NA			
			Total: 1250		**Total: 540**			**Total: 338**	**43.2**	**27.0**
VA	**580**	Corn	470	88	412	71	54	222		
		Soybean	580	25	145	25	87	126		
		Cotton	61	38	23	4	68	16		
			Total: 1111		**Total: 580**			**Total: 364**	**52.2**	**32.8**

The Eastern Coastal region summary (Table IX-10) was developed by compiling the data from all the states within the region. Unlike the individual state data, the data in Column G for this regional summary were obtained by dividing Column F by Column B and the data in Column H were obtained by dividing Column I by Column F. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2008 planting data (USDA-NASS, 2008); "other" crop and newly seeded alfalfa acreages are based on 2008 planting data from the Individual States data which were obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp.

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

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

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

[5] Roundup Ready rotational crop adoption rates for corn, soybean and cotton are based on 2008 planting data (USDA-NASS, 2009a). The percentages for Roundup Ready corn, cotton and soybean represent the percentages for total herbicide-tolerant traits. Percentages of herbicide-tolerant alfalfa and sugar beets are future market adoption estimates.

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

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

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

[9] Sweet corn and other vegetables.

[10] Sweet corn, and other vegetables.

[11] Sweet corn and onions.

[12] Cucumbers and sweet potatoes.

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 when the combine is improperly adjusted for efficient harvesting. Volunteer soybean in rotational crops is not a concern in the Midwest region because the soybean seed is typically not viable after the winter period (Carpenter et al., 2002; OECD, 2000). In southern soybean growing areas of the U.S. where the winter temperatures are milder, it is possible for soybean seed to remain viable over the winter and germinate the following spring.

Volunteer soybean normally is not a concern in rotational crops, such as corn, cotton, rice, and 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 postemergence herbicides also are available to control volunteer soybean (conventional or glyphosate tolerant soybean) in each of the major rotational crops. Table IX-11 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, postemergence applications of AAtrex (atrazine), Clarity (dicamba), Distinct (diflufenzopyr + dicamba), Hornet (flumetsulam + clopyralid) and Widematch (clopyralid + fluroxypyr) provide excellent control (Zollinger, 2005). In wheat, Bronate Advanced (bromoxynil), Clarity (dicamba) and Widematch postemergence provide excellent control of volunteer soybean (Zollinger, 2005).

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

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

Table IX-11. 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.

IX.I. Stewardship of MON 87705

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 by implementing product stewardship processes throughout the lifecycle of a product and by participation in the Excellence Through Stewardship[SM] Program (http://www.excellencethroughstewardship.org/). These policies and practices include rigorous field compliance and quality management systems and verification through auditing.

As with all of our products, Monsanto is committed to the rigorous product stewardship of MON 87705. In keeping with past practice, Monsanto will seek regulatory approval for MON 87705 in all key soybean import countries with a functioning regulatory system to assure global compliance and support the flow of international trade. Monsanto continues to monitor other countries that are key importers of soybean from the U. S., for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will make appropriate and timely regulatory submissions.

As with other value-added specialty soybean, once appropriate approvals are received, MON 87705-derived varieties will be grown using an appropriate IDP system based on established practices as described in Section IX.B.3. IDP practices are implemented for value-added specialty soybean to capture the enhanced value of the product and ensure that the end-user or processor receives the soybean with the identity, fatty acid composition of the oil, and desired quality.

Prior to obtaining all key global regulatory approvals, Monsanto will conduct seed testing, variety development and production, and oil manufacturing, testing and commercial activities leading to the commercial introduction of soybean varieties developed with MON 87705. Monsanto will work in a closed loop system under contract with partners to plant, harvest and process MON 87705 and MON 87705-derived soybean for the purpose of producing low saturated fat, high oleic oil.

A closed loop system will utilize appropriate processes for containment, documentation and traceability of seed production, planting, harvest, processing and use of the product. Grain production processes will include mass balance and accounting of all planting, harvested seed, secured storage facilities, labeling of all soybean, training of personnel, identification and audit of field production sites and acreage, spatial isolation of fields, equipment clean out procedures and documentation. Soybean processing will include segregation, control and traceability of handling, processing, packaging, and shipping of products and co-products.

Before implementing a closed loop system, Monsanto will dialogue with the appropriate value chain stakeholders in the countries of production and of use of the product to gain feedback on and confirm the robustness and validity of the closed loop production system. Monsanto will not employ the closed loop system without adequate assurance that the system will be effective in containing, preventing the escape of, and controlling

the disposition of MON 87705 and MON 87705-derived soybean so it is not comingled with commodity soybean. As part of this process, Monsanto will continue to provide regular updates on MON 87705 to key members of the soybean industry grain trade, processing industry and food industry throughout the regulatory and product development process.

IX.J. Impact of the Introduction of MON 87705 on Agricultural Practices

Introduction of MON 87705 is expected to have no impact on current cultivation and management practices for soybean. The Roundup Ready soybean system, i.e., 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 92% of U.S. soybean acres (USDA-NASS, 2008). Therefore from an agricultural perspective, MON 87705 is similar to the commercial Roundup Ready soybean products used in the U.S. since 1996. For an overview of the cumulative impacts on agricultural practices (weed control, tillage and crop rotation) from deregulation of glyphosate-tolerant crops see Appendix N.

Changes in the MON 87705 soybean oil profile are not anticipated to impact agricultural practices. MON 87705 is a nutritionally improved soybean product that is expected to bring added value to consumers that will result in the soybean being sold at a premium. The added value of MON 87705 as a specialty soybean is expected to bring higher returns to growers compared to commodity soybean. As such, MON 87705 will be harvested, processed and marketed using well-established IDP methods to maintain its integrity, purity and value.

X. ENVIRONMENTAL CONSEQUENCES AND IMPACT ON AGRONOMIC PRACTICES

X.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 87705 and its impact on agronomic practices. USDA-APHIS has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition can be granted, thereby allowing unrestricted introduction of the article.

The definition of "plant pest" in the Plant Protection Act (PPA) includes living organisms that could directly or indirectly injure, damage, or cause disease in any plant or plant product (7 U.S.C. § 7702[14]). Information in this petition related to plant pest risk characteristics includes the mechanism of action and changes to plant metabolism and composition, expression and characteristics of the gene products (CP4 EPSPS protein and the *FATB1-A* and *FAD2-1A* RNA), weediness of the regulated article, impacts to NTOs, disease and pest susceptibilities, 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 endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the regulated article is not likely to pose a plant pest risk. The plant pest risk assessment of MON 87705 is based primarily on eight lines of evidence: (1) insertion of a single functional copy of the inserted expression cassette, (2) characterization of the CP4 EPSPS protein expressed in MON 87705 and the improved fatty acid profile, (3) safety of the CP4 EPSPS protein and the improved fatty acid profile, (4) compositional equivalence of harvested MON 87705 soybean seed as compared to conventional soybean, (5) phenotypic and agronomic characteristics demonstrating no increased plant pest potential, (6) negligible risk to NTOs and threatened or endangered species, (7) modern soybean has inherently low plant pest potential, and (8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects than conventional soybean.

Phenotypic and agronomic characteristics of MON 87705 were analyzed to determine if it will become any more weedy or invasive relative to soybean varieties currently on the market approved for unconfined release. Additional evaluations included the propensity of MON 87705 to become a greater reservoir of plant pests (insects or pathogens) when compared to conventional plants. An assessment for potentially adverse impacts of MON 87705 on NTOs, including symbiotic soil microorganisms, also was performed. The potential for, and consequences of, gene flow and introgression of the genetic construct into sexually compatibile plants or wild relatives, were also evaluated to determine the potential for increased weedy or invasive characteristics in sexually compatible plant species.

Molecular characterization of MON 87705 by Southern blot analyses demonstrated that a single copy of the T-DNA I and T- DNA II sequences from the transformation vector PV-GMPQ/HT4404 was integrated into the soybean genome at a single locus. There were no additional genetic elements, including backbone sequences, from the transformation vector PV-GMPQ/HT4404 detected, linked or unlinked to the intact DNA insert, in MON 87705. The assessment of weediness potential and gene flow indicated that MON 87705 is no more likely to become a weed than conventional soybean, and MON 87705 is expected to 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 because the CP4 EPSPS protein which confers herbicide tolerance has no meaningful toxicity to humans and NTOs under the conditions of use. Transfer of the tandem *FATB1-A* and *FAD2-1A* gene segments is equally unlikely. As with the *cp4 epsps* gene, even if transfer were to occur, the result would be negligible because the *FATB1-A* and *FAD2-1A* gene segments do not encode for a protein, nucleic acids are generally recognized as safe (GRAS), and the resulting change in fatty acids results in an oil profile that is similar in composition to other plant-derived oils. An assessment of current agronomic practices in the U.S. indicates that the introduction of MON 87705 will not impact current U.S. soybean cultivation practices or the management of weeds, diseases, or insects (Section IX).

Using the assessment above, the data and analysis presented in this petition leads to a conclusion that MON 87705 is unlikely to be a plant pest and, therefore, no longer should be subject to regulation under 7 CFR §340.

APHIS recently has proposed amendments to 7 CFR § 340 that include its noxious weed authority. Because the data show that MON 87705 has no potential to cause injury or damage to protected interests under the noxious weed authority, MON 87705 also would not be considered a "noxious weed" as defined by the Plant Protection Act.

X.B. Plant Pest Assessment of the MON 87705 Insert and Expressed Substances

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

Genetic Insert

MON 87705 was produced by *Agrobacterium*-mediated transformation of soybean with PV-GMPQ/HT4404, which is a binary vector containing 2T-DNAs (Table IV-1). T-DNA I contains a *cp4 epsps* expression cassette and a partial suppression cassette containing the sense segments of the *FAD2-1A* intron and *FATB1-A* 5' UTR. T-DNA II contains a partial suppression cassette that consists of the antisense segments of *FAD2-1A* and *FATB1-A*. During plant transformation, the two T-DNAs co-integrated at one locus in the soybean genome, creating a single DNA insert that contains the single *cp4 epsps* cassette and a single *FAD2-1A* and *FATB1-A* suppression cassette. The chimeric promoter P-FMV/TSF1 consists of enhancer sequences from the 35S promoter of the

figwort mosaic virus (FMV) and the promoter from the Tsf1 gene of the plant *Arabidopsis thaliana* encoding elongation factor EF-1 alpha. The FMV promoter has a history of safe use in transgenic plants (USDA-APHIS, 2006b) and is highly unlikely to promote plant disease. The inserted T-DNA in MON 87705 contains left and right border sequences from *Agrobacterium tumefaciens*, a plant pest. These sequences are well characterized, are noncoding regions and will not cause MON 87705 to promote plant disease (Section IV).

Molecular analyses demonstrate that MON 87705 contains one copy of the insert at a single integration locus. No additional elements from the transformation vector were detected in the genome of MON 87705, including backbone sequence from plasmid PV-GMPQ/HT4404. 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 the expected CP4 EPSPS protein and dsRNA are produced from the inserted DNA.

Mechanism of Action

Monsanto Company has developed biotechnology-derived soybean with an improved soybean oil profile. Compared to conventional soybean oil, MON 87705 soybean oil fatty acid (FA) levels are lower for saturated fats (6% vs. 15% FA), and higher for oleic acid (76% vs. 23% FA) (USDA-NND database at http://www.nad.usda.gov). The increase in monounsaturated fatty acid (oleic) is accompanied by an overall decrease in polyunsaturated fatty acids (17% vs 60% FA). As described earlier in Section I.C., the improved fatty acid profile in MON 87705 soybean oil is achieved through the use of endogenous soybean (*Glycine max* L.) gene segments configured to suppress FATB and FAD2 gene expression. MON 87705 contains *FATB1-A* and *FAD2-1A* gene segments under the control of a seed promoter, limiting oil composition modification to this tissue. The assembled gene transcript has an inverted repeat that produces double stranded RNA (dsRNA) that, via the RNA interference (RNAi) pathway, suppresses endogenous FATB and FAD2 gene expression, thereby producing the desired fatty acid phenotype. Acyl-acyl carrier protein (ACP) thioesterases (referred to herein as FATB enzymes) are localized in plastids and hydrolyze saturated fatty acids from the ACP-fatty acid moiety. The suppression of FATB results in a decrease in the transport of the saturated fats out of the plastid, thus retaining their availability for desaturation to 18:1 oleic acid (see Figure E-1). Therefore, suppression of FATB decreases saturated fat content in the oil as well as increasing oleic acid. Subsequently, this increased amount of oleic acid is either delivered to the oil body or endoplasmic reticulum for further desaturation. Delta-12 desaturases (referred to as FAD2 enzymes) desaturate 18:1 oleic acid to 18:2 linoleic acid. The suppression of FAD2 in soybean seed causes reduced desaturation of oleic to linoleic acid thus contributing further to the increase in oleic while reducing linoleic acid content in the oil. Therefore, the overall result of the suppression of these two enzymes is a reduction in saturated 16:0 palmitic and 18:0 stearic fatty acids, an increase in monounsaturated 18:1 oleic acid, and lower levels of polyunsaturated 18:2 linoleic acid relative to commodity soybean.

The RNA-based suppression of *FATB* and *FAD2* soybean genes in MON 87705 is mediated by dsRNA molecules. Double stranded RNAs are commonly found in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids. Nucleic acids have a long history of safe consumption and are considered GRAS by the U.S. Food and Drug Administration. There is no evidence to suggest dietary consumption of RNA is associated with toxicity or allergenicity. Moreover, analysis of the DNA segments encoding this dsRNA showed that they lack the sequences required for translation initiation and protein synthesis. The production of a protein from the dsRNA encoded by the insert in MON 87705 is highly unlikely. Several biotechnology-derived plant products previously deregulated by APHIS were developed using RNA-based suppression mechanisms, including virus-resistant papaya and squash, high oleic soybean, FLAVR SAVR tomatoes, and plum trees resistant to Plum pox virus (FDA, 1994; USDA-APHIS 1994; USDA-APHIS, 1997; USDA-APHIS, 2006; USDA-APHIS, 2007). Based on this information, it is concluded that the inserted DNA and resulting dsRNA are safe and unlikely to produce a protein or polypeptide. As a result, the RNA-based suppression technology used in MON 87705 poses no novel risks from a food, feed or environmental perspective.

MON 87705 incorporates the *cp4 epsps* coding sequence derived from the common soil bacterium *Agrobacterium* sp. strain CP4. The *cp4 epsps* coding sequence directs the production of the 5-enolpyruvylshikimate-3-phosphate synthase (termed CP4 EPSPS protein) that is less sensitive to inhibition by glyphosate compared to endogenous EPSPS in plants. The CP4 EPSPS protein renders Roundup Ready soybean tolerant to glyphosate, the active ingredient in Roundup agricultural herbicides. The CP4 EPSPS protein has been assessed previously as a component in multiple Roundup Ready crops and has not been found to confer a selective advantage which would lead to increased plant pest risk.

Expressed Protein Safety

The CP4 EPSPS protein produced in MON 87705 is equivalent to the CP4 EPSPS proteins consumed in food and feed derived from other Roundup Ready crops, such as Roundup Ready soybean, that have a history of safe use. It is structurally homologous to EPSPS proteins that are part of the amino acid synthesis pathway of all plants (Devine et al., 1993). The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been extensively evaluated by Harrison et al. (1996). EPA also has reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR § 174.523). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of other Roundup Ready crops in 1996. Therefore, it is concluded that the CP4 EPSPS protein poses no risk to human or animal health.

Protein Expression Levels

CP4 EPSPS mean protein expression levels in MON 87705 samples range from 77 to 530 µg/g dry weight for root, forage, harvested seed and overseason leaf (see Section VI). These levels are comparable to other commercialized CP4 EPSPS protein-containing soybean products and confer tolerance to glyphosate found in the agricultural herbicide Roundup. The inverted repeat encoded by the insert in MON 87705 does not code for any proteins. Thus, no new proteins are produced in soybean containing the MON 87705 trait.

X.B.2. Compositional Characteristics

MON 87705 soybean seed was expected to be compositionally equivalent to conventional soybean except for the intended changes in their fatty acid composition brought about by the suppression of *FATB* and *FAD2* RNAs. Compositional analyses were conducted on seed and forage from MON 87705 samples collected at multiple sites. Additional non biotechnology-derived conventional soybean varieties also were included in the analysis to establish a range of natural variability for each analyte.

The compositional analyses confirmed MON 87705 seed had the intended change in fatty acid composition; that is, decreased levels of the saturated fatty acids, elevated levels of 18:1 oleic acid, and a corresponding decrease in 18:2 linoleic acid. Other components analyzed in MON 87705 seed and forage were compositionally equivalent to that of conventional soybean control (Section VII). Collectively, the data established that, with the exception of intended changes in fatty acid levels, MON 87705 is compositionally equivalent to conventional soybean.

Similarly, fatty acid analysis of MON 87705 refined, bleached and deodorized oil confirmed the intended differences for saturated fatty acids, oleic acid, and linoleic acid. Food and feed derived from MON 87705 soybean oil will be lower in saturated fats than food and feed derived from commodity soybean oil, and is considered nutritionally improved. Other components analyzed in MON 87705 processed fractions were compositionally equivalent to that of conventional soybean control. Collectively, the data established that, with the exception of intended changes in fatty acid levels, MON 87705 is compositionally equivalent to conventional soybean.

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

An extensive and robust set of information and data were used to assess whether the introduction of the improved oil profile trait altered the plant pest potential (OECD, 1993) of MON 87705 compared to the conventional soybean control, which had a genetic background similar to MON 87705 but did not possess the improved fatty acid profile trait. Phenotypic and agronomic characteristics of MON 87705 were evaluated and compared to those of the conventional soybean control. These assessments included seed dormancy and germination parameters, plant growth and development characteristics, plant responses to abiotic stressors, pollen morphology and viability and plant symbiont interactions (Section VIII). Results from the phenotypic and agronomic assessments demonstrate that MON 87705 does not possess characteristics that would confer a plant pest risk compared to conventional soybean. Data on environmental interactions also

indicate that MON 87705 does not confer any biologically meaningful increased susceptibility or tolerance to specific disease, insect, or abiotic stressors, or changes in agronomic and phenotypic characteristics. Taken together, these data support the conclusion that MON 87705 is not likely to pose increased plant pest risk compared to conventional soybean.

Seed Dormancy and Germination

Seed dormancy and germination characterization indicated that MON 87705 seed had germination characteristics similar to that of the conventional soybean control. In particular, the lack of hard seed, a well-accepted characteristic often associated with plants that are weeds, supports a conclusion of no increased weediness potential of MON 87705 when compared to conventional soybean.

Plant Growth and Development

Evaluations of plant growth and development characteristics in the field are useful for assessing potential weediness characteristics such as lodging and pod shattering, indicated there were no significant differences between MON 87705 and the conventional control. Of the remaining 12 growth and development characteristics assessed, there were four significant differences (early stand count, final stand count, days to flowering and 100 seed weight). The differences in these parameters were relatively small in magnitude, and the mean values of MON 87705 were within the range of the reference varieties for these characteristics. Thus, the differences in these parameters are unlikely to be biologically meaningful in terms of increased weed potential.

Response to Abiotic Stressors

Comparative field observations between MON 87705 and its conventional control and their response to abiotic stressors, such as drought, heat stress, high winds, nutrient deficiency, etc., found no differences. Therefore, these factors indicate no increased weediness potential.

Pollen Morphology and Viability

Evaluations of pollen morphology and viability from field-grown plants may be indicative of increased plant pest potential as they relate to the potential for gene flow to, and introgression of the biotechnology-derived trait into other soybean varieties and wild relatives. These evaluations demonstrated no statistically significant differences between MON 87705 and the conventional control. Taken together, these comparative assessments indicate that MON 87705 is not likely to have increased weed or plant pest potential compared to conventional soybean.

Interactions with NTOs

Evaluation of MON 87705 for potential adverse impacts on NTOs is a component of the plant pest risk assessment. The nature of MON 87705 as a product with no pesticidal activity leads to a conclusion that all exposed organisms are considered to be NTOs. In a 2007 U.S. phenotypic and agronomic study, observational data on environmental interactions were collected at select sites for MON 87705 and a conventional soybean control. In addition, multiple commercial conventional soybean varieties were included

in the analysis to establish a range of natural variability for each characteristic. The environmental interactions evaluation (Section VIII) included data collected on plant-insect, plant-disease, and plant-environment interactions. The results of this assessment indicated that the presence of the improved fatty acid profile and herbicide tolerance traits did not alter plant-insect interactions, including beneficial arthropods and insect pests, nor did it alter disease susceptibility of MON 87705 compared to conventional soybean. The lack of differences in plant response to disease damage, arthropod damage, and arthropod pest and beneficial insect abundance demonstrate that the introduction of the improved fatty acid profile trait is unlikely to be biologically meaningful in terms of increased pest potential.

In the field, soybean forms a complex symbiotic relationship with members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae*. This symbiosis results in the formation of root nodules in which the bacteria reduce or fix atmospheric nitrogen-producing ammonia that can be used by the plant. No significant differences were detected between MON 87705 and the control for the parameters measured, indicating no impact on either the symbiotic relationship or the symbiotic nitrogen-fixing bacteria. A lack of altered pest potential of MON 87705, compared to conventional soybean, is further supported by an assessment demonstrating that the symbiosis between nitrogen fixing bacteria and soybean was not altered as a result of the introduction of the improved oil profile and herbicide tolerance traits (Section VIII). Consequently, there is no increased plant pest potential due to the nutritionally improved soybean oil profile or herbicide tolerance.

These results provide evidence that when compared to conventional soybean, the environmental interactions between MON 87705 and arthropod pests, beneficial arthropods, diseases, and nitrogen-fixing bacteria were not altered. The lack of significant biological differences in plant response to environmental interactions indicates that it is unlikely the improved fatty acid profile and herbicide-tolerance traits of MON 87705 will be biologically meaningful in terms of increased plant pest potential.

X.C. Weediness Potential of MON 87705

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 § 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 potentially can grow as volunteer plants. However, volunteer plants likely would be killed by frost during autumn or winter of the year they were produced. If they did become established, volunteer plants would not compete well with the succeeding crop, and could be controlled readily by either mechanical or chemical means (OECD, 2000). In addition, since wild populations of *Glycine* species are not known to exist in the U.S., the potential

does not exist for MON 87705 to outcross to wild or weedy relatives and to alter weediness potential.

In comparative studies between MON 87705 and a conventional soybean control, phenotypic, agronomic, and environmental interaction data were evaluated (Section VIII) for changes that would impact the plant pest potential and, in particular, plant weediness potential. Results of these evaluations show that there is no fundamental difference between MON 87705 and the conventional control for traits potentially associated with weediness. Furthermore, comparative field observations between MON 87705 and its conventional control and their response to abiotic stressors, such as drought, heat stress, and high winds, indicated no differences and, therefore, no increased weediness potential. Collectively, these findings support the conclusion that MON 87705 has no increased weed potential compared to conventional soybean. Data on environmental interactions also indicate that MON 87705 does not confer any biologically meaningful increased susceptibility or tolerance to specific disease or insect stressors.

X.D. Potential for Pollen mediated Gene Flow

Gene introgression is a process whereby one or more genes successfully integrate into the genome of a recipient plant. Introgression is affected by many factors, including the frequency of the initial pollination event, environmental factors, sexual compatibility of pollen donor and recipient plants, pollination biology, flowering phenology, hybrid stability and fertility, selection, and the ability to backcross repeatedly. Because gene introgression is a natural biological process, it does not constitute an environmental risk in and of itself (Sutherland and Poppy, 2005). Gene introgression must be considered in the context of the transgenes inserted into the biotechnology-derived plant, and the likelihood that the presence of the transgenes and their subsequent transfer to recipient plants will result in increased plant pest potential. The potential for gene introgression from MON 87705 is discussed below.

X.D.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; Ray et al., 2003; Yoshimura et al., 2006). In studies with cultivated soybean where conditions have been optimized to ensure close proximity and flowering synchrony, natural cross-pollination generally has been found to be very low. Most outcrossing occurred with surrounding plants, and cross-pollination frequencies 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-1. Under natural conditions, cross-pollination among adjacent plants in a row or among plants in adjacent rows ranged from 0 to 6.3%. In experiments where supplemental pollinators (usually bees) were added to the experimental area, cross-pollination ranged from 0.5 to 7.74% in adjacent plants or adjacent rows. However, cross-pollination does not occur at these levels over long distances. Cross-pollination rates decrease to less than 1.5%

beyond one meter from the pollen source, and rapidly decrease with greater distances from the source. The following cross-pollination rates at extended distances have been reported: 0.02% at 8.2 m of separation (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, 2006a).

The ecological risk associated with gene flow and introgression from MON 87705 derives from the presence of the *cp4 epsps* gene and the *FAD2-1A* and *FATB1-A* gene segments. The consequence of introgression of the *cp4 epsps* gene and the *FAD2-1A* and *FATB1-A* gene segments from MON 87705 into other soybean is negligible since, as data presented in this petition confirm, they confer no increased plant pest potential to soybean.

X.D.2. Hybridization with Wild Annual Species within Subgenus Soja

The subgenus Soja includes the cultivated soybean *Glycine. max* and the wild annual species *Glycine soja*. *Glycine soja* is found in China, Taiwan, Japan, Korea, and Russia and can hybridize naturally with the cultivated soybean, *G. max* (Hymowitz, 2004). Hybridization between female *G. soja* and male *G. max* was less successful than 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). Abe et al. (1999) note that "natural hybrids between *G. max* and *G. soja* are rare … and hybrid swarms involving both species have never been reported." This is also supported by work from Kuroda et al (2008) in which molecular markers was used and no gene flow from *G. max* to *G.soja* was detected. Many barriers exist to natural hybridization between soybean and wild relatives, including the highly selfing nature of both plants, required proximity of wild soybean to cultivated soybean, synchrony of flowering, and presence of pollinators. As such, it is highly unlikely that naturally occurring, pollen-mediated gene flow and transgene introgression into wild soybean relatives from incidentally released biotechnology-derived soybean will occur at any meaningful frequency.

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 to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. *G. gracilis* may be 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). Although hybridization between *G. max* and members of the subgenus *G. soja* can take place, *G. soja* is not found in North or South America, and it is highly unlikely that gene transfer will occur.

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

Wild perennial species of the *Glycine* subgenus occur in Australia; West, Central and South Pacific Islands; China; Papua, New Guinea; Philippines; and Taiwan. 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 have been germinated aseptically *in vitro*, but the resulting F1 hybrids are slow-growing, morphologically weak, and completely sterile. Their sterility is due to poor chromosome pairing. Furthermore, species distantly related usually produce nonviable F1 seeds that either have premature death of the germinating seedlings or suffer from seedling and vegetative lethality (Kollipara et al., 1993; Singh and Hymowitz, 1989). In North and South America, it is not possible for gene transfer to occur between cultivated soybean and wild perennial species of *Glycine* subgenera because these wild species do not exist in these regions.

X.D.4. 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 sexually incompatible plant species. The likelihood for horizontal gene flow to occur is exceedingly small. The ecological risk associated with gene flow and introgression from MON 87705 derives from the presence of the *cp4 epsps* gene and the *FAD2-1A* and *FATB1-A* gene segments. The consequence of introgression of the *cp4 epsps* gene and the *FAD2-1A* and *FATB1-A* gene segments from MON 87705 into other soybean is negligible since, as data presented in this petition confirm, they confer no increased plant pest potential to soybean.

X.E. Summary of Plant Pest Assessments

Plant pests are defined in the Plant Protection Act as certain living organisms that can directly or indirectly injure, cause damage to, or cause disease to any plant or plant product (7 U.S.C. § 7702[14]). Characterization data presented in Sections III through IX of this petition confirms MON 87705 is not meaningfully different from conventional soybean in terms of pest potential in its phenotypic, agronomic and environmental interaction characteristics, with the exception of the nutritionally improved fatty acid profile and glyphosate tolerance. Thus, MON 87705 is similar to conventional soybean in its plant pest potential. Monsanto is not aware of any study results or observations associated with MON 87705 that would suggest an increased plant pest risk would result from its introduction.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of MON 87705 compared to conventional soybean, followed by a risk assessment on detected differences. The risk assessment considered various factors including: (1) insertion of a single functional copy of the inserted expression cassette, (2) characterization of the CP4 EPSPS protein expressed in MON 87705 and the improved

sfatty acid profile, (3) safety of the CP4 EPSPS protein and the improved fatty acid profile, (4) compositional equivalence of harvested MON 87705 soybean seed as compared to conventional soybean, (5) phenotypic and agronomic characteristics demonstrating no increased plant pest potential, (6) negligible risk to NTOs and threatened or endangered species, (7) modern soybean has inherently low plant pest potential, and (8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects than conventional soybean.

Based on the data and information presented in this petition, it is concluded that, like currently deregulated soybean, MON 87705 is highly unlikely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87705 and any progeny derived from crosses between MON 87705 and other commercial soybean be granted nonregulated status under 7 CFR § 340.

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

Distance from Pollen Source	% Cross-Pollination	Comments	Reference
0.3 m	0.04% (estimated per pod)	Interspaced plants within a row. Experiment conducted in a single year. Single male and female parental varieties. Percent outcrossing calculated per pod rather than per seed.	Woodworth, 1922
0.8 m	0.07 to 0.18%	Adjacent rows. Experiment conducted over two years. Several male and female parental varieties.	Garber and Odland, 1926
0.1 m	0.38 to 2.43%	Adjacent plants within a row. Experiment conducted in a single year. Several male and female parental varieties.	Cutler, 1934
0.1 m	0.2 to 1%	Adjacent plants within a row. Experiment conducted in single year at two locations. Several male and female parental varieties.	Weber and Hanson, 1961
0.9 m 2.7 – 4.6 m 6.4 – 8.2 m 10 – 15.5 m	0.03 to 0.44 % 0.007 to 0.04% 0 to 0.02% 0 to 0.01%	Frequency by distance was investigated. Experiment conducted over three years. Single male and female parental varieties.	Caviness, 1966
0.8 m	0.3 to 3.62%	Various arrangements within and among adjacent rows. Experiment conducted over three years. Several male and female parental varieties.	Beard and Knowles, 1971
One row (undefined)	1.15 to 7.74%	Bee pollination of single-row, small-plots of pollen receptor surrounded by large fields (several acres) of pollen donor soybean. Soybean is not a preferred flower for honeybee.	Abrams et al., 1978

Table X-1 (cont.) Summary of Published Literature on Soybean Cross Pollination

Distance from Pollen Source	% Cross-Pollination	Comments	Reference
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	0.65 to 6.32% 1.8%	Interspaced plants within a row. Experiment conducted in a single year. Single male and female parental varieties.	Ray et al., 2003
0.7 m 1.4 m 2.1 m 2.8 m 3.5 m 7.0 m 10.5 m	0 to 0.19% 0 to 0.04% 0 to 0.05% 0 to 0.08% 0 to 0.04% 0 to 0.04% 0	Interspaced plants within a row arranged in small plots. Experiment conducted in a four year period. Single male and two female parental varieties.	Yoshimura, et al., 2006

XI. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto knows of no study results or observations associated with MON 87705, the improved fatty acid profile or the CP4 EPSPS protein, indicating that there would be an adverse environmental consequence from the introduction of MON 87705. MON 87705 soybean oil contains a reduced level of saturated fats, an increase in oleic acid and an associated decrease in linoleic acid levels. The decrease in saturated fats and polyunsaturated fatty acids in MON 87705 soybean oil provides important options for food companies to develop foods with lower saturated fat and greater food functionality. In addition, these attributes provide key enhancements for biodiesel and industrial applications. MON 87705 also contains the CP4 EPSPS protein that renders the soybean plant tolerant to glyphosate, the active ingredient in the Roundup family of agricultural herbicides. As demonstrated by field results and laboratory tests, the only phenotypic difference between MON 87705 and conventional soybean is the improved soybean fatty acid profile and glyphosate tolerance.

The data and information presented in this petition demonstrate that MON 87705 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 the *cp4 epsps* and partial suppression cassette at a single locus within the soybean genome; that the suppression of *FATB* and *FAD2* RNAs result in the improved soybean fatty acid profile, and that the CP4 EPSPS protein was expressed in tissues at levels that resulted in tolerance to the herbicide glyphosate. Extensive characterization of the plant phenotype, including compositional analysis of key nutrients and antinutrients also indicated MON 87705, with the exception of the intended fatty acid changes, was unchanged compared to conventional soybean. A history of safe use of the CP4 EPSPS protein is supported by the lack of any documented reports of adverse effects since the introduction of other Roundup Ready crops in 1996. The EPA previously reviewed and established a tolerance exemption for the CP4 EPSPS protein and the genetic material necessary for its production in or on all raw agricultural commodities.

An endangered species risk assessment concluded MON 87705 is unlikely to have adverse effects on these organisms. Therefore, the risks for humans, animals, and other NTOs from MON 87705 are negligible under the conditions of use. Additionally the introduction of MON 87705 will not adversely impact cultivation practices or the management of weeds, diseases, and insects in soybean production systems.

Successful adoption of MON 87705 will provide growers with an opportunity to produce this value-added specialty soybean that produces soybean oil with improved stability and a healthier profile to help meet needs in food and industrial markets.

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APPENDICES

Appendix A. USDA Notification

Field trials of MON 87705 were conducted in the U.S. since 2005. 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 87705, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2007-2008 seasons, are still in preparation. A list of trials conducted under USDA notification and the status of the final reports for these trials are provided in Table A-1.

Table A-1. USDA Notifications Approved for MON 87705 and Status of Trials Conducted under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
2005			
05-220-03n	9/28/2005	PR	Submitted to USDA
05-242-05n	9/28/2005	HI	Submitted to USDA
2006			
06-033-05n	5/18/2006	HI(3)	Submitted to USDA
06-045-13n	5/18/2006	HI(5)	Submitted to USDA
06-045-18n	5/18/2006	PR(3)	Submitted to USDA
06-109-04n	6/14/2006	PR(3)	Submitted to USDA
06-201-102n	9/11/2006	IA, PR(2)	Submitted to USDA
06-033-01n	3/14/2006	IL(7), KS(5)	Submitted to USDA
06-033-02n	3/23/2006	IA(7), IL(5), IN(2)	Submitted to USDA
06-223-111n	9/11/2006	PR(4)	Submitted to USDA
06-319-102n	12/15/2006	PR(3)	Submitted to USDA
2007			
07-038-117n	4/9/2007	IA(2)	Submitted to USDA
07-046-108n	4/1/2007	IL(2)	Submitted to USDA
07-054-103n	3/25/2007	IA, IL(2), NE, OH	Submitted to USDA
07-043-104n	3/20/2007	IA(2), IL(2), IN(2), MO, NE, OH, PA, WI	Submitted to USDA
07-094-105n	5/4/2007	WI	Submitted to USDA
07-043-105n	4/17/2007	AR(2), IL, IN, KS, MI, MO(5)	Submitted to USDA
07-137-104n	6/16/2007	PR(2)	Submitted to USDA
07-247-108n	10/4/2007	PR(2)	Submitted to USDA
07-211-102n	8/31/2007	PR(2)	Submitted to USDA
07-254-103n	10/11/2007	IA, IL, IN, MO, NE	Submitted to USDA
07-031-109n	3/21/2007	IL(10), IN(3), MO	Submitted to USDA
07-031-112n	3/20/2007	AR, IA(7), KS(6), MD	Submitted to USDA
07-352-101rm	3/26/2008	IA(8), IL(16), IN(4), KS(6)	Submitted to USDA
07-031-102n	3/18/2007	PR(2)	Submitted to USDA
07-032-101n	4/5/2007	IL(3)	Submitted to USDA

Table A-1 (cont.) USDA Notifications Approved for MON 87705 and Status of Trials Conducted under These Notifications

2008			
08-056-109n	3/28/2008	AR, IL(3), MD, WI	Submitted to USDA
08-058-113n	3/28/2008	IA(2), IL(2), IN(2), OH, PR(2)	Submitted to USDA
08-045-118n	3/15/2008	PR(2)	Submitted to USDA
08-049-101n	3/19/2008	IL, MD, WI	Submitted to USDA
08-058-105n	3/28/2008	IL, IN, MI, MO, WI(2)	Submitted to USDA
08-080-114n	4/19/2008	IA, PR(2)	Submitted to USDA
08-079-101n	4/17/2008	IA(3)	Submitted to USDA
08-084-102n	4/24/2008	IA, NE	Submitted to USDA
08-137-101n	6/15/2008	PR	Submitted to USDA
08-170-102n	7/18/2008	PR(2)	Submitted to USDA
08-182-101n	8/1/2008	PR(2)	Submitted to USDA
08-270-101n	10/26/2008	PR	In Progress
08-301-103n	11/26/2008	HI, PR	In Progress
08-357-101rm	3/17/2009	IA(11), IL(14), KS(5), IN(3), MO, NE	In Progress
08-323-101n	12/18/2008	PR(3)	In Progress
09-007-106n	2/25/2009	PR(2)	In Progress
08-042-101n	3/14/2008	AR, IA, IL(2), IN, MI, NE(2)	Submitted to USDA
08-042-102n	3/12/2008	IA(2), IL(8), IN(4), MO	Submitted to USDA
08-042-107n	3/12/2008	IA(7), KS(5)	Submitted to USDA
2009			
09-050-117n	3/21/2009	HI, IA(4), IL(4), IN(2), OH(2), PR, WI	In Progress
09-033-101n	3/1/2009	IA(8), KS(5), NE	In Progress
09-061-108n	4/1/2009	AR, IA, IL(5), IN(2), KS, MI, MO, NE, WI(2)	In Progress
09-050-134n	3/21/2009	HI, PR	In Progress
09-068-110n	4/8/2009	IL(5)	In Progress
09-099-102n	5/9/2009	PR(2)	In Progress
09-124-105n	5/13/2009	IA	In Progress
09-135-103n	6/14/2009	IL	In Progress
09-135-104n	6/14/2009	IL	In Progress
09-030-105n	3/1/2009	IA(3), IL(4), IN, KS, NE	In Progress
09-030-104n	3/1/2009	IA(3), IL(8), IN(3), MO	In Progress
09-036-103n	3/7/2009	IA(2), IL(2), IN, MS, NE(2)	In Progress

Appendix B. Materials and Methods Used for Molecular Analyses of MON 87705

B.1 Materials

The DNA used in molecular analyses was isolated from leaf tissue of MON 87705 collected in 2008 harvested from Production Plan 07-01-83-30 (Seed lot: GLP 0704 18620-S). Additional DNA extracted from various MON 87705 generations of leaf tissues were used in generation stability analyses. The control substance was conventional soybean variety A3525 which has the same genetic background as the test substance. The reference substance, plasmid PV-GMPQ/HT4404 (Figures IV-1 and 2), was used as the transformation vector to develop MON 87705. The plasmid was digested and used as a positive hybridization control in Southern analyses. Probe templates generated from this plasmid also served as positive hybridization controls. As additional reference standards, the 1 kb DNA extension ladder and λ DNA/Hind III segments from Invitrogen (Carlsbad, CA) were used for size estimations on Southern blots and agarose gels. The 500 bp ladder from Invitrogen and GeneRuler 1 kb Plus Ladder from Fermentas (Hanover, MD) were used for size estimations for PCR analyses.

B.2 Characterization of the Materials

The quality of the source materials from MON 87705 and A3525 were verified by PCR analysis to confirm the presence or absence of MON 87705 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.

B.3 DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA from the test and control substances was extracted prior to the initiation of the study using a hexadecyltrimethylammonium bromide (CTAB)-based method according to SOP BR-ME-1153-01. DNA extractions were stored in a 4 °C refrigerator or a -20 °C freezer.

B.4 Quantification of Genomic DNA

Extracted genomic DNA and plasmid DNA were quantified prior to the initiation of the study using Hoefer's DyNA Quant 200 Fluorometer according to SOP BR-EQ-0065-02. Molecular size marker IX (Roche, Indianapolis, IN) was used as the DNA calibration standard.

B.5 Restriction Enzyme Digestion of Genomic DNA

Approximately 10 or 20 µg of genomic DNA extracted from the test and control substances were used for restriction enzyme digestions. When digesting genomic DNA with *Nco* I (New England BioLabs, Beverly, MA), 10X NE buffer 3 (New England BioLabs) was used. When digesting genomic DNA with the Spe I (Roche, Indianapolis, IN), 10X Tango buffer (Fermentas) was used. All digests were performed at 37°C according to SOP BR-ME-0316-01 in a total volume of ~500 µl using ~25-100 units of the appropriate restriction enzyme(s).

B.6 DNA Probe Preparation for Southern Blot Analyses

Probe template DNA containing sequences of plasmid PV-GMPQ/HT4404 was prepared by PCR amplification according to SOP BR-ME-0486-01 and gel purified according to SOP BR-ME-0889-01. The probes were designed based on the nucleotide content (%GC) so that the entire probe would be hybridized under the conditions appropriate for the sequence. Approximately 25 ng of each probe template were radiolabeled with ^{32}P-deoxyadenosine triphosphate (dATP) (6000 Ci/mmol) using the random priming method (RadPrime DNA Labeling System, Invitrogen) according to SOP BR-ME-0611-01. Approximately 1×10^6 cpm of labeled probe per ml of hybridization solution was hybridized to the Southern blot. Probe locations relative to the genetic elements in plasmid PV-GMPQ/HT4404 are depicted in Figures IV-1 and IV-2.

B.7 Southern Blot Analyses of Genomic DNA

Digested genomic DNA isolated from test and control material, in addition to predigested control material mixed with appropriate positive hybridization controls were evaluated using Southern blot analyses according to SOP BR-ME-0317-02. The plasmid DNA was digested and then added to the predigested conventional soybean genomic DNA to serve as a positive hybridization control. Probe templates were added to predigested control material to serve as an additional positive hybridization control. The DNA was then separated by agarose gel electrophoresis. Southern blots were hybridized and washed at 55° C or 60° C depending on the calculated melting temperature (T_m) of the probes used. The table below lists the hybridization 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	Element Sequence Spanned by DNA Probe	Hybridization/Wash Temperature (°C)
1	T-DNA I Probe 1A	Left Border + P-*FMV/Tsf1* + L-*Tsf1* + I-*Tsf1* (portion)	55
2	T-DNA I Probe 2B	I-*Tsf1* (portion) + TS-*CTP2* (portion)	55
3	T-DNA I Probe 3C	TS-*CTP2* (portion) + CS-*cp4 epsps* (portion)	60
4	T-DNA I Probe 4D	CS-*cp4 epsps* (portion) + T-*E9* + P-*7Sα'* + *FAD2-1A* (portion)	55
5	T-DNA I Probe 5E	*FAD2-1A*^{p} (portion)+ *FATB1-A*^{p} (portion) + Right Border	55
6	T-DNA II Probe 1A	T-*H6* + *FAD2-1A*^{p} + *FATB1-A*^{p}	55
7	Backbone Probe 1	Backbone Sequence	60
8	Backbone Probe 2	Backbone Sequence	60
9	Backbone Probe 3	Backbone Sequence	60
10	Backbone Probe 4	Backbone Sequence	60

B.8 DNA Sequence Analyses of the Insert

Overlapping PCR products were generated that span the insert in MON 87705. These products were sequenced to determine the nucleotide sequence of the insert in MON 87705, 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 according to SOP BR-ME-0486-01.

The PCR analyses were conducted using ~96-100 ng of genomic DNA template or ~12 ng of plasmid DNA in a 50 µl reaction volume or ~48-50 ng of genomic DNA template in a 25 µl reaction volume containing a final concentration of 1 mM MgSO4, 1 M Betaine, 0.8 µM of each primer, 0.2 mM each dNTP, and 0.02 units of KOD Hot Start DNA polymerase from Novagen (Gibbstown, NJ). The amplification of Products A, D, and E was performed under the following cycling conditions: 94°C for 2 minutes; 35 cycles at 94° C for 45 seconds, 65° C for 45 seconds, 72° C for 5 minutes; one cycle at 72°C for 10 minutes. The amplification of Products B, C, and F was performed under the following cycling conditions: 94° C for 2 minutes; 35 cycles at 94° C for 55 seconds, 68°C for 60 seconds, 72° C for 5 minutes; and one cycle at 72° C for 10 minutes.

Aliquots of each PCR product were separated on 0.8% (w/v) agarose E-gel® (Invitrogen) or separated on a 0.8% agarose gel according to SOP BR-ME-0315-02. Prior to sequencing, the PCR products were visualized by ethidium staining to verify the products were of the expected size prior to sequencing. To remove excess primers following PCR amplification, products were treated with a mixture of 0.1 unit Exonuclease I, designated as EXO, from USB (Cleveland, OH) and 0.1 unit Shrimp Alkaline Phosphatase, designated as SAP (USB) per 5 µl of PCR product and cycled at the following conditions: one cycle at 37° C for 15 minutes, one cycle at 80° C for 15 minutes. As documented in the raw data, not all products were treated with EXO-SAP prior to sequencing. The PCR products were sequenced using multiple primers, including primers used for PCR amplification. In addition, primers internal to the amplified PCR

sequences were used to sequence the regions of the amplicon. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry (ABI, Foster City, CA).

B.9 PCR and DNA Sequence Analysis to Examine the MON 87705 Insertion Site

To demonstrate that the DNA sequences flanking the insert in MON 87705 are native to the soybean genome and to examine the MON 87705 insertion site in conventional soybean, PCR and sequence analyses were performed on genomic DNA from both MON 87705 and conventional soybean. The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 87705. One primer designed from the genomic DNA sequence flanking the 5' end of the insert was paired with a second primer located in the genomic DNA sequence flanking the 3' end of the insert. The PCR analysis was performed according to SOP BR-ME-0486-01.

The PCR analyses were conducted using ~96-100 ng of genomic DNA template in a 50 µl reaction volume or ~50 ng of genomic DNA template in a 25 µl reaction volume containing a final concentration of 1 mM MgSO4, 1 M Betaine, 0.8 µM of each primer, 0.2 mM each dNTP, and 0.02 units of KOD Hot Start DNA polymerase (Novagen). The amplification of the product was performed under the following cycling conditions: 94°C for 2 minutes; 35 cycles at 94°C for 55 seconds, 68°C for 60 seconds, 72°C for 5 minutes; one cycle at 72°C for 10 minutes. Aliquots of each PCR product were separated on 0.8% (w/v) agarose E-gel® (Invitrogen) and visualized using the UV transilluminator to verify that the products were of the expected size prior to sequencing. To remove excess primer following

PCR amplification, the PCR product containing the conventional soybean template was treated with a mixture of 0.1 unit EXO and 0.1 unit SAP per 5µl of PCR product and cycled as follows: one cycle at 37°C for 15 minutes, one cycle at 80°C for 15 minutes. The PCR products were sequenced using multiple primers, including primers used for PCR amplification. In addition, primers internal to the amplified PCR sequences were used to sequence the regions of the amplicon. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry (ABI).

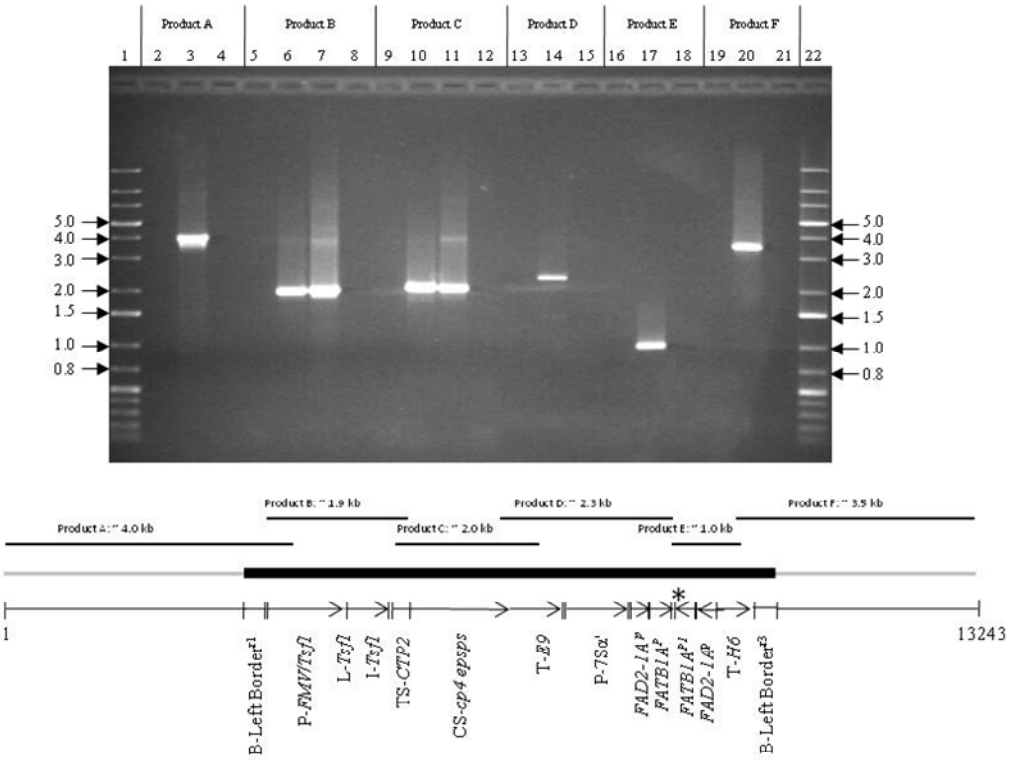

Figure B-1. Overlapping PCR Analysis across the Insert in MON 87705

PCR analyses demonstrating the linkage of the individual genetic elements within the insert in MON 87705 were performed on MON 87705 genomic DNA extracted from leaf (Lanes 3, 6, 10, 14, 17, and 20). Lanes 2, 5, 9, 13, 16, and 19 contain reactions with conventional soybean DNA while lanes 4, 8, 12, 15, 18, and 21 are reactions containing no template DNA. Lanes 7 and 11 contain reactions with PV-GMPQ/HT4404 plasmid control DNA. Lanes 1and 22 contain Fermentas GeneRuler 1 kb Plus DNA Ladder. Lanes are marked to show which product has been loaded and is visualized on the agarose gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 87705 that appears near the bottom of the figure. Three to fifteen microliters of each of the PCR products was loaded on the gel. This figure is representative of the data generated in the study; however the PCR amplicons reported in this figure were not necessarily used in sequencing.

Lane
1: GeneRuler™ 1 kb Plus DNA Ladder
2: Conventional soybean DNA
3: MON 87705 genomic DNA
4: No template DNA control
5: Conventional soybean DNA
6: MON 87705 genomic DNA
7: PV-GMPQ/HT4404 control DNA
8: No template DNA control
9: Conventional soybean DNA
10: MON 87705 genomic DNA
11: PV-GMPQ/HT4404 control DNA
12: No template DNA control
13: Conventional soybean DNA
14: MON 87705 genomic DNA
15: No template DNA control
16: Conventional soybean DNA
17: MON 87705 genomic DNA
18: No template DNA control
19: Conventional soybean DNA
20: MON 87705 genomic DNA
21: No template DNA control
22: GeneRuler 1 kb Plus DNA Ladder

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

* Symbol denotes partial sequences from Left and Right Border after integration into MON 87705.

Appendix C. Materials, Methods and Results for Characterization of CP4 EPSPS Protein Produced in MON 87705

C.1 Materials

The MON 87705-produced CP4 EPSPS protein (Orion lot 10002253) was purified as described below from harvested seed of MON 87705 prior to the initiation of this study. The identity of the harvested seed containing MON 87705 was confirmed by event-specific PCR; a copy of the Certificate of Analysis (COA) for this seed lot is archived in the Monsanto Regulatory archives with the records documenting protein isolation. The purified MON 87705-produced protein was stored in a -80 ºC freezer in a buffer solution containing 50 mM Tris-HCl, pH 7.5, 50mM KCl, 2mM DTT, 1mM benzamidine-HCl and 25% glycerol. The records describing the purification of this MON 87705-produced protein are archived under the Orion lot 10002253.

The *E. coli*-produced CP4 EPSPS reference protein (Orion lot 10000739) was purified from the fermentation of *E. coli* transformed with a plasmid containing the *cp4 epsps* gene. The DNA sequence encoding this CP4 EPSPS 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 10000739. The *E. coli*-produced CP4 EPSPS protein reference standard was previously characterized (APS Characterization Plan 20-100015) and a copy of the COA is included in the Monsanto archives. The *E. coli*-produced CP4 EPSPS protein was stored in a -80 °C freezer in a buffer solution (50 mM Tris-Cl, 1 mM benzamidine-HCl, 50 mM KCl, 2 mM DTT, and 25% (v:v) glycerol, pH 7.5) at a total protein concentration of 3.8 mg/ml with a purity of 97%.

The *E. coli*-produced CP4 EPSPS 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.

C.2 Description of Assay Controls

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 BSA standard (BioRad, Hercules, CA) were used to generate a standard curve for determining total 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.

C.3 Protein Purification

The plant-produced CP4 EPSPS protein was purified from seed of MON 87705 prior to initiation of this characterization plan. The purification procedure was not performed under a GLP plan; however, all procedures were documented on worksheets and, where

applicable, SOPs were followed. The CP4 EPSPS protein was purified from an extract of ground seed using a combination of ammonium sulfate fractionation, hydrophobic interaction chromatography, anion exchange chromatography, and cellulose phosphate affinity chromatography. All protein extraction and chromatography steps were performed at ~4°C. A detailed description of the purification process was filed under Orion Lot 10002253, and is briefly described below.

Approximately 100 g of pre-chilled seed of MON 87705 were ground using a Perten Laboratory Mill 3100. The ground powder (~ 100 g) was defatted 3 times with 500 ml each of Hexanes (w:v, EMD, Gibbstown, NJ) prewarmed to 37 ºC, air-dried, and stored in a -80 ºC freezer prior to extraction of the CP4 EPSPS protein. The portion of the defatted seed powder (50 g) was mixed with an extraction buffer (100 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM Benzamidine-HCl, 4 mM DTT, 2 mM Phenylmethylsulfonyl fluoride, 1% Polyvinylpolypyrrolidone and 10% glycerol) for 2 hours at approximately a 1:10 powder weight to extraction buffer volume ratio. The slurry was centrifuged at 23,500 × g for 20 minutes at ~ 4 ºC. The resultant 430 ml supernatant was subjected to 40% ammonium sulfate protein fractionation by addition of 97 g of ammonium sulfate over one hour in the cold room (2 ºC to 8 ºC). The solution was stirred for two hours at ~ 4 ºC and centrifuged at 23,500 X g for 20 minutes. Another 89.8 g of ammonium sulfate was added to the supernatant (480 ml) over a period of one hour to 70% saturation. The solution was stirred for two hours in a 4 ºC cold room and the pellet was collected by centrifugation at 23,500 X g for 30 min. The pellet was resuspended in 150 ml of buffer designed as PS(A) [50 mM Tris-HCl, pH 7.5, 1 mM DTT, 10% glycerol (v:v), 1.5 M $(NH_4)2SO_4$]. The resuspended sample was loaded onto a 140 ml column of Phenyl Sepharose Fast Flow (5 cm X 7 cm column) (GE Healthcare, Piscataway, NJ) equilibrated with the buffer PS(A). Proteins were eluted with a linear salt gradient that decreased from 1.5 to 0 M $(NH_4)2SO_4$ in the buffer PS(A) over a volume of 1400 ml. Fractions containing the CP4 EPSPS protein, identified based on western blot analysis, were pooled to a final volume of ~250 ml. The pooled sample was desalted by dialysis against 4 L of buffer designed as QS(A) (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM benzamidine-HCl, 4 mM DTT) for 20 h at ~4 °C (with three additional buffer changes) using dialysis tubing [Molecular Weight Cutoff (MWCO), 12 to 14 kDa] (Spectrum Laboratories, Inc. Rancho Dominguez, CA).

The desalted sample (337 ml) was loaded onto a 60 ml column of Q Sepharose Fast Flow anion exchange resin column (2 cm X 20 cm) (GE Healthcare, Piscataway, NJ), which was equilibrated with the QS(A) buffer. Bound CP4 EPSPS protein was eluted with a linear salt gradient that increased from 0 M to 0.4 M potassium chloride in the QS(A) buffer over 600 ml. Fractions containing CP4 EPSPS protein, identified by western blot analysis, were pooled to a final volume of ~ 140 ml. The pooled sample was place into dialysis tubing (MWCO, 12 to 14 kDa, Spectrum Laboratories, Inc. Rancho Dominguez, CA) and dialyzed against buffer designed as CP(A) [50 mM MES, pH 5.8, 10% glycerol (v:v), 1 mM benzamidine-HCl and 1 mM DTT] for 18 hours at ~4 °C in three buffer changes.

The dialyzed sample (100 ml) was then loaded onto a 19 ml cellulose phosphate P11 cation exchange column (1.6 X 9.5 cm) pre-equilibrated with the CP(A) buffer. Bound CP4 EPSPS protein was eluted with the CP(A) buffer containing 0.5 mM

phosphoenolpyruvate (PEP) and 0.5 mM S3P. Fractions containing CP4 EPSPS protein, based on SDS PAGE analysis, were pooled (~14.5 ml). This pooled sample was concentrated to 10 ml at ~4 °C using a slide-A-lyzer dialysis cassette (MWCO: 10 kDa, size: 3 to 12 ml, Pierce, Rockford, IL) and covering it in a water absorbing polymer powder (Aquacide I, EMD, Gibbstown, NJ). After concentration, the cassette was placed into 2 L of dialysis buffer (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine-HCl) and dialyzed for a total of 20 hours at ~ 4 °C in three 2 L buffer changes. The dialyzed sample in the cassette was further concentrated to 5 ml using Aquacide I as described above. This 5 ml sample was mixed with 5 ml dialysis buffer containing 50% glycerol to final volume of 10 ml. Final buffer composition of the sample was 50 mM Tris-HCl, pH 7.5, 50mM KCl, 2mM DTT, 1 mM benzamidine-HCl and 25% glycerol. This CP4 EPSPS protein purified from seed of MON 87705 was aliquoted (100 µl each), assigned APS lot 10002253 and stored at ~ -80 °C.

C.4 Molecular Weight and Purity Estimation-SDS-PAGE

SDS-PAGE analysis was performed to determine the molecular weight of CP4 EPSPS protein purified from MON 87705 and to compare the molecular weight of the MON 87705- produced and *E coli*-produced CP4 EPSPS proteins.

An aliquot of the test substance was mixed with 5 X sample loading buffer (LB) [312 mM Tris-HCl, 20% (v:v) 2-mercaptoethanol, 10% (w:v) SDS, 0.025% (w:v) bromophenol blue, 50% (v:v) glycerol, pH 6.8)], to a final total protein concentration of 0.168 µg/µl. Molecular weight markers (Bio-Rad broad-range) and reference substance were diluted to a final total protein concentration of 0.9 and 0.2 µg/µl, respectively. The test substance was analyzed in duplicate at 1, 2, and 3 µg protein per lane. The *E. coli*-produced CP4 EPSPS protein reference standard (Orion lot 10000739) was analyzed at 1 µg total protein. All samples were heated at ~ 100 °C for three minutes and loaded onto a pre-cast Tris glycine 4 to 20% polyacrylamide gradient 10-well mini-gel (Invitrogen, Carlsbad, CA). Electrophoresis was performed at a constant voltage of 150 V for 95 minutes. Proteins were fixed by placing the gel in a solution of 40% (v:v) methanol and 7% (v:v) acetic acid for 30 minutes, stained for 18 hours and 35 minutes with Brilliant Blue G-Colloidal stain (Sigma-Aldrich, St. Louis, MO), destained 30 seconds with a solution containing 10% (v:v) acetic acid and 25% (v:v) methanol, and finally destained with 25% (v:v) methanol for 6.5 hours. Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). Molecular weight markers were used to estimate the apparent molecular weight of each observed band. All visible bands within each lane were quantified using Quantity One software. Apparent molecular weight and purity were reported as an average of all six loadings containing the MON 87705-produced CP4 EPSPS protein.

C.5 Immunoblot Analysis-Immunoreactivity

Immunoblot analysis was performed to confirm the identity of the CP4 EPSPS protein purified from MON 87705 and to compare the immunoreactivity of the MON 87705-produced and *E coli*-produced CP4 EPSPS proteins.

The MON 87705- produced and *E. coli*-produced CP4 EPSPS proteins were both loaded onto the same gel at equal loads of 1, 2, and 3 ng per well. Aliquots of each protein were diluted in water and 5 X LB heated at 100.5 °C for 3 min, and applied to a pre-cast Tris

glycine 4 to 20% polyacrylamide gradient 15-well gel (Invitrogen, Carlsbad, CA). Three amounts of each protein were loaded in duplicate on the gel. Electrophoresis was performed at a constant voltage of 150 V for 88 minutes. Pre-stained molecular weight markers (SeeBlue Plus2 Prestained, Invitrogen, Carlsbad, CA) were loaded in parallel to verify electrotransfer of the proteins to the membrane and estimate the size of the immunoreactive bands observed. Electrotransfer to a 0.45 µm nitrocellulose membrane (Invitrogen, Carlsbad, CA) was performed for 90 minutes at a constant voltage of 25 V.

For immunodetection, the membrane was blocked for 1 hour with 5% (w:v) Non-Fat Dried Milk (NFDM) in 1 X Phosphate Buffered Saline containing 0.05% (v:v) Tween-20 (PBST). The membrane was then probed with a 1:1,000 dilution of goat anti-CP4 EPSPS antibody (lot 10000787, aliquot # 20) in 5% (w:v) NFDM in PBST for one hour. Excess antibody was removed using three 10 minutes washes with PBST. Finally, the membrane was probed with horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG (Thermo, Rockford, IL) at a dilution of 1:10,000 in 5% (w:v) NFDM in PBST for one hour. Excess HRP-conjugate was removed using three 10 minutes washes with PBST. All incubations were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (GE, Healthcare, Piscataway, NJ) and exposed (10, 30, and 60 s) to Amersham Hyperfilm (GE, Healthcare, Piscataway, NJ). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

The immunoreactive bands of the MON 87705-produced CP4 EPSPS protein in each lane migrating to the same position as the reference standard were quantified and compared to the signals corresponding to the *E. coli* CP4 EPSPS protein reference substance. Quantification of the blot was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) using the lane finding and contour tool. The raw data was exported to a Microsoft Excel [2007 (12.0.6324.5001) SP1 MSO (12.0.6320.5000] file for the pair wise comparison of all the loads. An average absolute difference was calculated for each comparison to determine the immunoreactivity equivalence.

C.6 MALDI-TOF Tryptic Mass Map Analysis

MALDI-TOF MS was used to confirm the identity of the MON 87705-produced CP4 EPSPS protein. Since the protein was determined to be pure (100%) based on pre-study data, it was not deemed necessary to separate the protein by SDS-PAGE prior to trypsinization.

An ethanol precipitation was performed to concentrate the MON 87705-produced CP4 EPSPS protein sample and remove any buffer components that may interfere with the analysis. Twenty five µl of the MON 87705-produced CP4 EPSPS sample (0.21 mg/ml) was concentrated to approximately 20 µl with a Speed-Vac concentrator and then mixed with 200 µl prechilled 95% ethanol. After overnight incubation at -20 °C, the mixture was centrifuged at 13,000 X g for 30 minutes at ~4 °C. The pellet was collected, washed twice with 200 µl of prechilled acetone and then twice with 200 µl of water. Ten µl of trypsin solution [20 µg/ml trypsin (Promega, Madison, WI) in a 25 mM ammonium bicarbonate buffer, pH 7.8] was added and incubated overnight at 37 °C. Trypsin digested samples (0.3 µl) were added directly onto the analysis plate in triplicate and followed by the addition of ~ 0.75 µl of three matrices, 2,5-dihydroxybenzoic acid

(DHB), α-cyano-4-hydroxycinnamic acid (α-Cyano) , and Sinapinic acid (Waters Corp., Milford, MA) on separate spots. The sample in DHB matrix was analyzed in the 300 to 7500 dalton range using 100 shots at a laser intensity setting of 2480 (a unit-less MALDI-TOF instrument specific value) while samples in α-Cyano and Sinapinic acid were analyzed in the 500 to 7500 dalton range using 100 shots at a laser intensity setting of 1980 and 2380, respectively. Protonated (MH+) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). Calmix 2 was used as the external calibrant (Sequazyme Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) for the analysis. GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical trypsin digest of the CP4 EPSPS protein sequence based upon the nucleotide sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Experimental masses (MH+) were assigned to peaks in the 300 to 7500 Da range if they met the following criteria: resolved monoisotopic peak; with at least one additional associated ion peak for masses < 1000 Da and at least two associated ion peaks for masses > 1000 Da; peak height greater than twice the baseline noise; and did not overlap with a stronger mass signal (±2 daltons from the mass analyzed). Known autocatalytic segments from trypsin digestion were identified in the raw data. The list of experimental masses was then compared to the theoretical list from the GPMAW software. Those experimental masses within one Da of a theoretical mass were matched. All matching masses were tallied and a coverage map was generated. The tryptic mass map coverage was considered acceptable if ≥ 40 % of the protein sequence was identified by matching experimental masses observed for the tryptic peptide segments to the expected masses for the segments.

C.7 MALDI-TOF Mass Analysis of MON 87705-produced CP4 EPSPS Protein

MALDI-TOF mass spectrometry was used to confirm the molecular weight of the MON 87705-produced CP4 EPSPS protein. Since the protein was determined to be pure (100%) prior to this analysis, it was not deemed necessary to separate the protein by SDS-PAGE.

MALDI-TOF MS was used to further characterize the molecular weight of the MON 87705-produced CP4 EPSPS protein. Prior to analysis, the MON 87705-produced CP4 EPSPS protein was dialyzed using drop dialysis (Görisch, 1988). Briefly, a 25 mm Millipore microdialysis disk (type VSWP, 0.025 µm pore size, Bedford, MA) was floated on HPLC-grade water, spotted with 2 µl of the sample, and dialyzed for 45 minutes. A portion of the MON 87705-produced CP4 EPSPS and BSA protein samples (0.125 and 0.25 µl) was spotted on an analysis plate, mixed with 0.375 and 0.75 µl of 3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic acid) solution, respectively, and air-dried. Mass spectral analysis of the MON 87705-produced CP4 EPSPS protein was performed using an Applied Biosystems Voyager DE-Pro Biospectrometry Workstation MALDI-TOF instrument with the supplied Data Explorer software (version 4.0, Foster City, CA). Mass calibration of the instrument was performed using a BSA protein standard. The sample was analyzed in the 10,000 to 100,000 dalton range using 100 shots at a laser intensity setting of 2983 (a unit-less MALDI-TOF instrument specific value). Average protonated (MH+) protein masses were observed in linear mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23, Foster City,

CA) was used to generate a theoretical mass of the expected CP4 EPSPS protein sequence based upon the nucleotide sequence. The mass of the MON 87705-produced CP4 EPSPS protein was reported as an average of three separate mass spectral acquisitions.

C.8 N-Terminal Sequencing

N-terminal sequencing using automated Edman degradation chemistry was used to confirm the identity of the MON 87705-produced CP4 EPSPS protein.

Because the protein was determined to be 100% pure based on pre-study data, it was not necessary to separate the protein by SDS-PAGE. Therefore, an aliquot of the MON 87705-produced CP4 EPSPS protein was used for N-terminal sequence analysis. The analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785 Programmable Absorbance Detector and Procise Control Software (version 1.1a) were used. Chromatographic data were collected using Atlas99 software (version 3.59a, LabSystems, Altrincham, Cheshire, England). 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 (10 picomoles of β-lactoglobulin, Applied Biosystems, Foster City, CA) was analyzed before and after the analysis of the CP4 EPSPS protein to verify that the sequencer met performance criteria for repetitive yield and sequence identity. Identity was deemed to be established if ≥8 amino acids, consistent with the predicted sequence of the N-terminus of the MON 87705-produced CP4 EPSPS protein were observed during analysis.

C.9 Glycosylation Analysis

Glycosylation analysis was performed to determine whether the MON 87705-produced CP4 EPSPS protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 87705-produced CP4 EPSPS protein, the *E.-coli*-produced CP4 EPSPS reference standard protein, and the positive controls, transferrin (~ 76 – 81kDa, Sigma-Aldrich, St. Louis, MO), and horseradish peroxidase (~ 40 kDa, Pierce, Rockford, IL) were each diluted with water and mixed with 5× LB. These samples were heated at 97.6 °C for 5 minutes, cooled, and loaded on a Tris glycine 4-20% polyacrylamide gradient 10-well mini-gel. Each sample was loaded at 50 and 100 ng per lane. SeeBlue Plus2 pre-stained protein molecular weight markers (Invitrogen, Carlsbad, CA) were loaded to verify electrotransfer of the proteins to the membrane and the CandyCane Glycoprotein Molecular Weight Standards (Molecular Probes, Eugene, OR) were loaded as positive/negative controls and markers for molecular weight. Electrophoresis was performed at a constant voltage of 150 V for 80 minutes. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 90 minutes at a constant voltage of 25 V.

Carbohydrate detection was performed directly on the PVDF membrane using the Pro-Q Emerald 488 Glycoprotein Gel and Blot Stain Kit (Molecular Probes). The manufacturer's protocol was followed. All steps were performed at room temperature. The PVDF membrane was fixed in 25 ml of a solution containing 50% methanol and 5%

glacial acetic acid for one hour, the solution was then changed and the membrane was incubated overnight. Two, 15 minutes washes (50 ml each) with 3% (v:v) glacial acetic acid (wash solution) were followed by a 20 minutes oxidation in 25 ml of an oxidizing solution containing periodic acid (Component C from kit). The membrane was washed three times, 10 minutes each, in 50 ml of wash solution. The membrane was then incubated in 25 ml of Pro-Q Emerald Staining Solution that was prepared using the kit reagents. After 40 minutes of staining in the dark, one 15 minutes, 50 ml wash cycle was followed by two 30 minutes, 50 ml wash cycles. The final wash cycles included two 50 ml, one minute deionized water washes followed by three five minutes methanol washes(EMD, San Diego, CA). The blot was then scanned using the BioRad Molecular Imager FX using the Alexa 488 illumination setting (Quantity One software; version 4.6, build 036) in order to visualize the fluorescently labeled glycosylated proteins.

After glycosylation analysis the blot was stained to visualize the proteins present on the membrane. Proteins were stained for one minute using Coomassie Brilliant Blue R-250 staining solution (Bio-Rad, Hercules, CA) and then destained with 1× destain solution (Bio-Rad, Hercules, CA) for 5 min. After washing with water, the blot was scanned using Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) in order to visualize total proteins.

C.10 Functional Activity Assay

In order to assess the functional activity of the MON 87705-produced CP4 EPSPS protein and to compare its activity to the *E. coli*-produced CP4 EPSPS reference standard protein, aliquots of the MON 87705-produced CP4 EPSPS protein and *E. coli*-produced CP4 EPSPS reference standard protein were analyzed for their ability to release inorganic phosphate from phosphoenolpyruvate (PEP). The specific activity is expressed in units per mg of protein (U/mg), where a unit (U) is defined as 1 μmole of inorganic phosphate released from PEP per minute at 25 °C.

The assay was carried out on a micro titer plate. Prior to functional activity analysis, both test and reference proteins were diluted to a purity corrected concentration of 50 μg/ml with a 50 mM HEPES, pH 7.0 buffer. Assays for both proteins were conducted in triplicate. Each assay replicate was subsequently analyzed spectrophotometrically in duplicate. Briefly, the reactions containing the CP4 EPSPS enzyme with S3P were initiated by the addition of PEP. The reactions were performed in a mixture of 50 mM HEPES (pH 7.0), 0.1 mM ammonium molybdate, 2 mM S3P, 1 mM PEP and 5 mM potassium fluoride for two minutes at 25.2 °C. The reactions were quenched with malachite green (phosphate assay reagent) and fixed after two minutes with 33% (w:v) sodium citrate. The release of inorganic phosphate from PEP was determined at a wavelength of 660 nm using a PowerWave Xi (Bio-Tek, Richmond, VA) microplate reader, and quantitated relative to a standard curve of inorganic phosphate treated with the malachite green (phosphate assay) reagent and 33% (w:v) sodium citrate.

C.11 Results of CP4 EPSPS Protein Molecular Weight Equivalence

The equivalence in apparent molecular weight of the purified MON 87705-produced and the *E. coli*-produced CP4 EPSPS protein was demonstrated using SDS-PAGE (Figure C-1). The MON 87705-produced CP4 EPSPS protein migrated with a molecular weight

indistinguishable to that of the *E. coli*-produced protein standard analyzed concurrently (Table C-1). Based on comparable electrophoretic mobilities, the MON 87705-produced and *E.coli*-produced CP4 EPSPS proteins were determined to have equivalent apparent molecular weights.

The predicted mass of the MON 87705-produced CP4 EPSPS protein was also confirmed by MALDI-TOF MS. The average mass obtained for CP4 EPSPS was 47,396 Da. This experimentally obtained mass differs from the theoretical mass calculated for the CP4 EPSPS reference standard protein minus the N-terminal methionine by only 0.18%. The difference between the expected and the observed mass for MON 87705-produced CP4 EPSPS is minimal and within the acceptable error for MALDI-TOF mass determination. The absence of the N-terminal methionine was confirmed by N-terminal sequencing.

Table C-1. Molecular Weight Difference Between the MON 87705- and *E. coli* - produced CP4 EPSPS Proteins.

Molecular Weight of MON 87705-Produced CP4 EPSPS Protein[1]	Molecular Weight of *E. coli*-produced CP4 EPSPS Protein[2]	% Difference from *E. coli* - produced CP4 EPSPS Protein[3]
44.6 kDa	43.8 kDa	1.8%

[1] Reference **Table C-1** for the molecular weight of the full-length MON 87705-produced protein.
[2] Reference the Orion 10000739 COA for the molecular weight of the full-length *E. coli* - produced reference protein.
[3] Percent difference was calculated as follows: $\frac{44.6 - 43.8}{44.6} \times 100\ \% = 1.8\%$

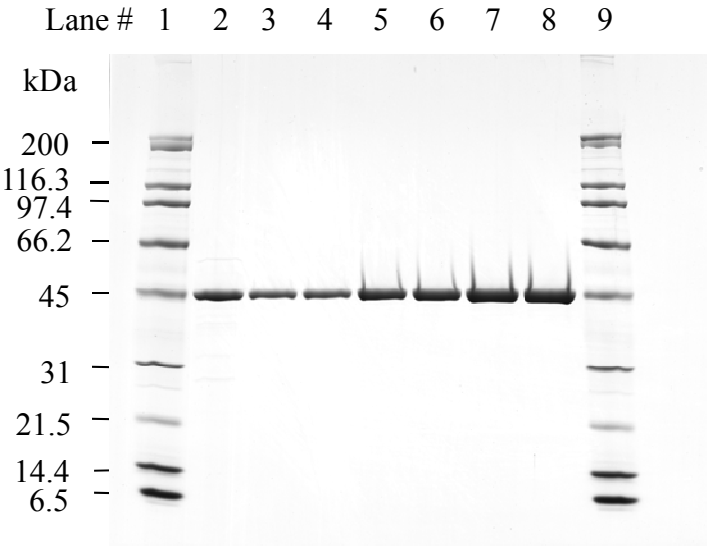

Figure C-1. Molecular Weight and Purity Analysis of the MON 87705-Produced CP4 EPSPS Protein
Aliquots of the MON 87705- produced and the *E. coli*-produced CP4 EPSPS proteins were separated on a 4 to 20% Tris glycine polyacrylamide gradient gel and then stained with Brilliant Blue G-Colloidal stain. Approximate MWs (kDa) are shown on the left and correspond to the markers loaded in Lanes 1 and 9.

Lane	Sample	Amount loaded (µg)
1	Broad Range molecular weight markers	4.5
2	*E. coli* -produced CP4 EPSPS protein standard	1
3	MON 87705-produced CP4 EPSPS protein	1
4	MON 87705-produced CP4 EPSPS protein	1
5	MON 87705-produced CP4 EPSPS protein	2
6	MON 87705-produced CP4 EPSPS protein	2
7	MON 87705-produced CP4 EPSPS protein	3
8	MON 87705-produced CP4 EPSPS protein	3
9	Broad Range molecular weight markers	4.5

C.12 Results of CP4 EPSPS Protein Immunoreactivity Equivalence

A western blot analysis using goat anti-CP4 EPSPS serum was conducted to determine the relative immunoreactivity of the MON 87705-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS protein reference standard. The results demonstrated that the anti-CP4 EPSPS antibody recognized the MON 87705-produced CP4 EPSPS protein that migrated to a similar position as the *E. coli*-produced reference standard protein (Figure C-2). Furthermore, the immunoreactive signal increased with increasing levels of CP4 EPSPS protein loaded. The observed immunoreactivities between the MON 87705-produced and *E. coli*-produced proteins were similar based on densitometric analysis of the western blot (Table C-2). Based on the above analysis, the MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins demonstrated equivalent immunoreactive properties, which confirmed the identity and equivalence of the two proteins.

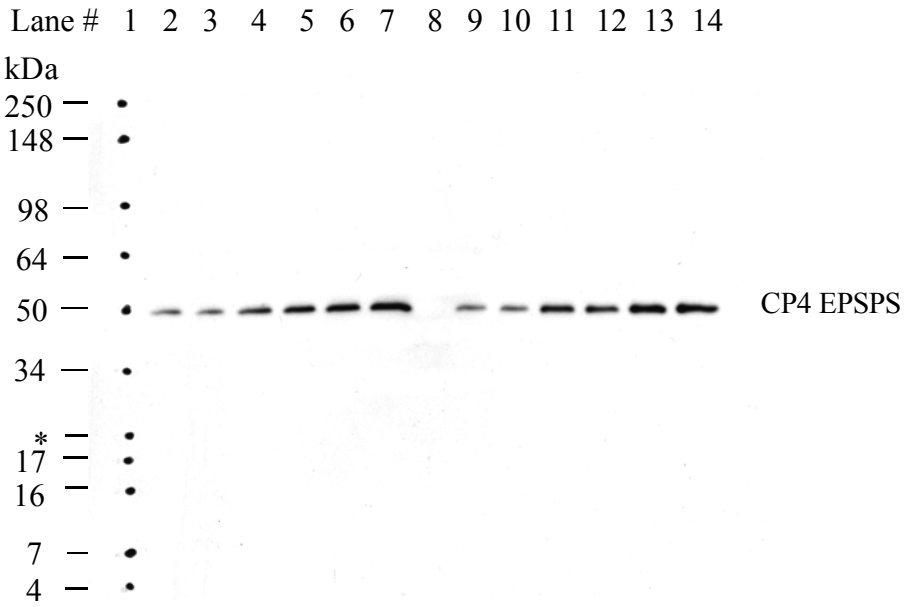

Figure C-2. Western Blot Analysis of MON 87705-produced and *E. coli* -produced CP4 EPSPS Proteins

Aliquots of the purified, MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins were separated by SDS-PAGE, and electrotransferred to a PVDF membrane. The membrane was probed with goat anti-CP4 EPSPS antibodies and developed using an ECL system (GE Healthcare). Approximate molecular weights (kDa) of markers loaded in Lane 1 are shown on the left side of the blot. The 30 second exposure is shown. *: Non-assigned molecular weight in marker.

Lane	Sample	Amount Loaded (ng)
1	See Blue® Plus2 Pre-Stained molecular weight markers	-
2	*E. coli* -produced CP4 EPSPS reference standard	1
3	*E. coli* -produced CP4 EPSPS reference standard	1
4	*E. coli*- produced CP4 EPSPS reference standard	2
5	*E. coli* -produced CP4 EPSPS reference standard	2
6	*E. coli* -produced CP4 EPSPS reference standard	3
7	*E. coli* -produced CP4 EPSPS reference standard	3
8	Empty	
9	MON 87705-produced CP4 EPSPS protein	1
10	MON 87705-produced CP4 EPSPS protein	1
11	MON 87705-produced CP4 EPSPS protein	2
12	MON 87705-produced CP4 EPSPS protein	2
13	MON 87705-produced CP4 EPSPS protein	3
14	MON 87705-produced CP4 EPSPS protein	3

Table C-2. Comparison of Immunoreactive Signal between MON 87705-produced and *E. coli* -produced CP4 EPSPS Proteins

Sample	Gel lane	Amount (ng)	Density (OD x mm^2)	Average Density[1]	Percent Difference[2] (%)	Average Difference[3] (%)
E. coli CP4 EPSPS	2	1	0.994	0.957	9.8	10.5 ± 1.8
E. coli CP4 EPSPS	3	1	0.920			
				1.062		
MON 87705 CP4 EPSPS	9	1	1.027			
MON 87705 CP4 EPSPS	10	1	1.096			
				2.163	13.0	
E. coli CP4 EPSPS	4	2	1.904			
E. coli CP4 EPSPS	5	2	2.421			
				2.485		
MON 87705 CP4 EPSPS	11	2	2.584			
MON 87705 CP4 EPSPS	12	2	2.386			
				3.766	8.7	
E. coli CP4 EPSPS	6	3	3.296			
E. coli CP4 EPSPS	7	3	4.236			
				4.124		
MON 87705 CP4 EPSPS	13	3	4.039			
MON 87705 CP4 EPSPS	14	3	4.208			

[1]Average Density =∑〖Density〗/2

[2]Percent Difference (%) $= \frac{|\text{Average Density plant} - \text{Average Density E.coli}|}{\text{Average Density plant}}$ X 100%

[3]Average difference (%) =∑〖% difference〗/3. The standard deviation was calculated using Microsoft Office Excel 2007 (12.0.6324.5001) SP1 MSO (12.0.6320.5000).

C.13 Results of MALDI-TOF Tryptic Mass Map Analysis

The MON 87705-produced, CP4 EPSPS protein was assessed by MALDI-TOF MS. 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 segments to expected (theoretical) mass segments. In general, 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. Observed tryptic peptides were considered a match to the expected tryptic mass when differences in molecular weight of less than one dalton (Da) were found between the observed and predicted segment masses. Such matches were made without consideration for potential natural amino acid modifications such as glycosylation. The protein sample was digested with trypsin and the masses of the tryptic peptides were measured.

There were 30 unique peptide segments (Table C-3), identified that corresponded to the expected masses of the CP4 EPSPS trypsin-digested peptides. The identified masses were used to assemble a coverage map indicating the matched peptide sequences for the entire CP4 EPSPS protein (Figure C-3), resulting in ~80% (362 out of 455 amino acids) coverage of the total protein. This analysis confirmed the identity of the MON 87705-produced CP4 EPSPS protein.

Table C-3. Summary of the Tryptic Masses Identified for the MON 87705-Produced CP4 EPSPS Using MALDI-TOF MS.

Matrix			Expected Mass[1]	Difference[2]	AA position[3]	Segment
α-Cyano	DHB	Sinapinic acid				
	389.18		*389.25*	0.07	225-227	TIR
	416.23		416.30	0.07	70-72	IRK
	474.20		474.27	0.07	228-231	LEGR
	506.17		506.22	0.05	354-357	ESDR
599.31	599.27		599.33	0.02	29-33	SISHR
616.32	616.29	616.10	616.34	0.02	128-132	RPMGR
629.32	629.28		629.29	0.03	201-205	DHTEK
629.32	629.28		629.34	0.02	383-388	GRPDGK
711.45	711.42		711.45	0	133-138	VLNPLR
835.39	835.37	835.29	835.39	0	62-69	AMQAMGAR
863.46	863.44		863.46	0	15-23	SSGLSGTVR
872.45	872.43		872.45	0	313-320	GVTVPEDR
872.45	872.43		872.52	0.07	358-366	LSAVANGLK
948.52	948.50		948.52	0	161-168	TPTPITYR
991.56			991.55	0.01	14-23	KSSGLSGTVR
1115.58	1115.58		1115.57	0.01	295-305	LAGGEDVADLR
1357.73	1357.73		1357.71	0.02	146-157	SEDGDRLPVTLR
1359.67	1359.69	1359.54	1359.72	0.05	354-366	ESDRLSAVANGLK
1359.67	1359.69	1359.54	1359.64	0.03	34-46	SFMFGGLASGETR
	1558.90	1558.73	1558.83	0.07	47-61	ITGLLEGEDVINTGK
1646.86	1646.89		1646.84	0.02	389-405	GLGNASGAAVATHLDHR
1705.82	1705.88		1705.81	0.01	367-382	LNGVDCDEGETSLVVR
1994.03	1994.07	1993.82	1993.97	0.06	206-224	MLQGFGANLTVETDADGVR
2183.24	2183.30	2183.05	2183.17	0.07	275-294	TGLILTLQEMGADIEVINPR
2367.43	2367.50	2367.21	2367.33	0.10	178-200	SAVLLAGLNTPGITTVIEPIMTR
		2450.13	2450.23	0.10	24-46	IPGDKSISHRSFMFGGLASGETR
		2450.13	2450.22	0.09	105-127	LTMGLVGVYDFDSTFIGDASLTK
	3186.35	3186.30	3186.52	0.17	73-104	EGDTWIIDGVGNGGLLAPEAPLDFGNAATGCR
	3249.77	3249.46	3249.62	0.15	321-351	APSMIDEYPILAVAAAFAEGATVMNGLEELR
		4188.82	4188.26	0.56	234-274	LTGQVIDVPGDPSSTAFPLVAALLVPGSDVTILNVLMNPTR

[1]Only experimental masses that matched expected masses are listed in the table.
[2]The numbers represent the difference between the expected mass and the first column which has the corresponding numbers.
[3]AA position refers to amino acid position within the predicted CP4 EPSPS sequence as depicted in Figure C-3.

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001 MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL

051 LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA

101 TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD

151 RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR

201 DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF

251 PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED

301 VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL

351 RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT

401 HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS

451 DTKAA

Figure C-3. MALDI-TOF MS Coverage Map of the MON 87705-produced CP4 EPSPS.

The amino acid sequence of the CP4 EPSPS protein was deduced from the *cp4 epsps* gene present in MON 87705. Boxed regions correspond to tryptic peptides that were identified from the MON 87705-produced CP4 EPSPS protein sample using MALDI-TOF MS. In total, 79.6% (362 of 455 total amino acids) of the expected protein sequence was identified.

C.14 Results of N-terminal Sequence Analysis

N-terminal sequencing of the first 10 amino acids performed on MON 87705-produced CP4 EPSPS protein resulted in the sequence expected for the CP4 EPSPS protein (Table C-4) with the exception of the N-terminal methionine, which was not detected. This result is expected as removal of the N-terminal methionine, catalyzed by methionine aminopeptidase, is a common modification that occurs co-translationally before completion of the nascent protein chain and has no effect on protein structure or activity. The N-terminal sequence information, therefore, confirms the identity of the CP4 EPSPS protein isolated from MON 87705 and the intactness of its N-terminus.

Table C-4. N-Terminal Sequence of the MON 87705-produced CP4 EPSPS.

Amino acid residue # from the N-terminus	→	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Expected Sequence[1]	→	M	L	H	G	A	S	S	R	P	A	T
		|	|	|	|	|	|	|	|	|	|	|
Experimental Sequence	→	-	L	H	G	A	S	S	R	P	A	T

The expected amino acid sequence of the N-terminus of the CP4 EPSPS protein was deduced from the *cp4 epsps* gene present in soybean MON 87705. The experimental sequence obtained from the MON 87705-produced CP4 EPSPS was compared to the expected sequence. [1] The single letter IUPAC-IUB amino acid code is **M**, methionine; **L**, Leucine; **H,** histidine; **G**, glycine; **A**, alanine, **S**, serine; **R**, Arginine; **P**, proline; **T**, threonine; and (-) Indicates the **M** residue not observed.

C.15 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 assess whether potential post-translational glycosylation of the MON 87705-produced CP4 EPSPS protein occurred, the purified protein sample was subjected to glycosylation analysis. The *E. coli*-produced CP4 EPSPS reference standard represented a negative control. The positive controls were the transferrin and horseradish peroxidase (HRP) proteins which are known to have multiple covalently linked carbohydrate modifications. The transferrin protein and HRP, as well as the purified CP4 EPSPS protein isolated from MON 87705 and *E. coli* were separated on SDS-PAGE,

transferred to a PVDF membrane, and glycosylation analysis was performed to detect carbohydrate moieties on the proteins. The results of this analysis are shown in Figure C-4. The positive controls, transferrin and HRP, were detected at the expected molecular weights of ~75 and ~50 kDa, respectively, in a concentration-dependent manner (Figure C-4, Panel A, Lanes 4-5 and 2-3). A very faint signal, slightly above background, was observed for the MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins (Figure C-4, Panel A, Lanes 6-7 and 8-9). This low level signal could be due to low level oxidation of amino acid residues of the protein and/or nonspecific binding of florescent reagents. Further evidence that the signals observed for MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins are nonspecific are the following: (1) The faint signal was also associated with the *E. coli*-produced CP4 EPSPS could not be the product of a glycan moiety because *E. coli*-produced proteins are not glycosylated. (2) Mass spectrometry data demonstrated the absence of glycosylation of the MON 87705-produced CP4 EPSPS. Glycosylation would result in an increase in the protein mass relative to the theoretically calculated mass. No increase in protein mass was observed for the MON 87705-produced CP4 EPSPS protein determined by MALDI-TOF MS (47396 Da) as compared to its theoretical mass (47481.48 Da) (3) Four potential glycosylation sites can be identified in the amino acid sequence of the CP4 EPSPS protein: one O linked at T248 and three N-linked at N213, N271 and N392 (see Figure C-3 for amino acid positions). The tryptic segments containing these amino acids were identified for the MON 87705-produced CP4 EPSPS protein by MALDI-TOF mass spectrometry. All identified masses matched the expected non-modified peptide masses, indicating that no glycosylation had occurred.

To confirm that sufficient MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins were present for carbohydrate detection and glycosylation analysis, the membrane was stained with Coomassie Blue R250 stain to detect proteins (Figure C-4, Panel B). Both MON 87705-produced and *E. coli*-produced CP4 EPSPS were clearly detected on the membrane (Figure C-4, Panel B, Lanes 6-9).

These results indicate that the MON 87705-produced CP4 EPSPS protein is not glycosylated and, thus is equivalent to the *E. coli*-produced CP4 EPSPS reference standard.

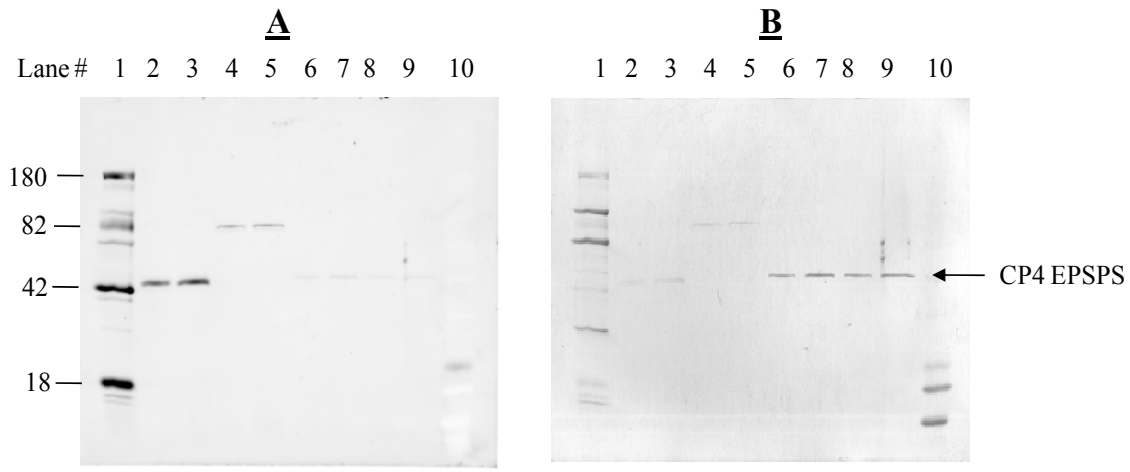

Figure C- 4. Glycosylation Analysis of the MON 87705-Produced CP4 EPSPS Protein

Aliquots of the MON 87705-produced CP4 EPSPS protein, *E. coli*-produced CP4 EPSPS reference standard (negative control), horseradish peroxidase (positive control) and transferrin (positive control) were separated by SDS-PAGE (4-20% gradient) and electrotransferred to a PVDF membrane. (**A**) Where present, periodate-oxidized protein-bound carbohydrate moieties reacted with Pro-Q Emerald 488 glycoprotein stain and emitted a fluorescent signal at 488 nm (Lanes 1-5). (**B**) The same blot was stained with Coomassie Blue R250. The signal was captured using a Bio-Rad GS-800 scanner. Approximate molecular weights (kDa) correspond to the CandyCane glycosylated markers loaded in Lane 1

Lane	Sample	Amount (ng)
1	CandyCane Glycoprotein molecular weight standards	—
2	Horseradish Peroxidase (positive control)	50
3	Horseradish Peroxidase (positive control)	100
4	Transferrin (positive control)	50
5	Transferrin (positive control)	100
6	MON 87705-produced CP4 EPSPS	50
7	MON 87705-produced CP4 EPSPS	100
8	*E. coli*-produced CP4 EPSPS (negative control)	50
9	*E. coli*-produced CP4 EPSPS (negative control)	100
10	See Blue® Plus2 Pre-Stained molecular weight markers	—

C.16 Results of Functional Activity

The functional activities of the MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins were estimated using an assay that measures the EPSPS-catalyzed formation of inorganic phosphate (P_i) and 5-enolpyruvylshikimate-3-phosphate (EPSP) from Shikimate-3-phosphate (S3P) and Phosphoenolpyruvate (PEP). In this assay protein specific activity is expressed as units per milligram of protein (U/mg), where a unit is defined as one μmole of inorganic phosphate released from PEP per minute at 25 °C. The MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins were considered functionally equivalent if the specific activity of one protein was within 50% (assay variability) of the other.

Results indicated that the specific activity was 4.10 U/mg protein for the MON 87705-produced CP4 EPSPS, and 4.38 U/mg protein for the *E. coli*-produced reference standard. The difference in specific activities was 6.4% (Table C-5) which is within assay variability. These results clearly demonstrate that the CP4 EPSPS proteins derived from MON 87705 and *E. coli* have equivalent functional activities.

Table C-5. CP4 EPSPS Functional Assay

The specific activity of the plant-produced CP4 EPSPS protein was determined using a phosphate release assay. This end-point type colorimetric assay measures the release of inorganic phosphate from one of the substrates, PEP, by the action of the CP4 EPSPS enzyme.

MON 87705-produced *CP4 EPSPS*[1] *(U/mg)*	*E. coli*-produced *CP4 EPSPS*[1] *(U/mg)*	*Difference (%, Plant vs E. coli)*[2]
4.10 ± 0.1	4.38 ± 0.33	6.4

[1] Value refers to mean and standard deviation calculated based on n= 6

[2] $\%\ \text{Difference} = \frac{(\text{Activity } E.coli - \text{Activity MON 87705})}{\text{Activity } E.coli} \times 100\%$

Appendix C References

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Appendix D. Materials and Methods Used for the Analysis of the Levels of CP4 EPSPS Protein in MON 87705

D.1 Materials

Tissue samples analyzed in this study were produced from five field sites in Chile during the 2007-2008 planting season from seed lot GLP-0702-18254-S for MON 87705 and GLP-0702-18252-S for the conventional control. The conventional control, A3525, does not contain the *cp4 epsps* coding region. Samples were stored in a -80 °C freezer throughout the study. An *E. coli*-produced CP4 EPSPS protein (Monsanto APS lot 20-100015) was used as a reference standard for the assay.

D.2 Characterization of the Materials

The identities of the MON 87705 and conventional control substances were confirmed by analysis of the harvested seed DNA by an event-specific polymerase chain reaction (PCR) method and the resulting Verifications of Identity (VOIs) were archived in the Monsanto Regulatory Archives. The copies of VOIs were also included in the study file.

D.3 Field Design and Tissue Collection

Production Plan REG-07-170 was initiated during the 2007-2008 planting season to generate MON 87705 and conventional control substances at various soybean-growing locations in Chile. The field sites were as follows: Quilapilum, Chacabuco; Melipilla, Melipilla; Calera de Tango, Maipo; Rancagua, Cachapoal; and San Fernando, Colchagua. 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 87705 and the conventional control were planted using a randomized complete block field design. Throughout the field production sample identity was maintained by using unique sample identifiers and proper chain-of-custody documentation. All tissue samples, except seed, were stored in a -80 ºC freezer and shipped on dry ice to the Monsanto processing facility in St. Louis, Missouri. 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 V2-V3 growth stage; OSL2 at V7; OSL3 at V10; and OSL4 at V14. The root and forage tissues were collected at approximately the R5-R6 growth stage, and the above-ground portion of the plant was labelled as the forage, and the below ground portion was washed and labelled as root tissue. Harvested seed samples were collected at the R8 growth stage.

D.4 Tissue Processing and Protein Extraction

All tissue samples produced at the field sites were shipped to Monsanto's processing facility. 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 50 ml tubes and stored in a -80°C freezer until use.

The CP4 EPSPS protein was extracted from soybean tissues as described in Table D-1. The CP4 EPSPS protein was extracted from all leaf, forage, root and seed tissues using a Harbil Mixer with 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 all tissue extracts using a serum filter (Fisher Scientific, Pittsburgh, PA). The extracts were aliquotted and stored frozen in a -80°C freezer until ELISA analysis.

Table D-1. Protein Extraction Methods for Tissue Samples

Tissue	Extraction Buffer	T:B Ratio	No. of ¼ in Chrome-steel beads	Shake time (minutes)	Sample Clarification method
Leaf[1]	TBA	1:50	8	3.5	Serum Filter
Root	TBA	1:50	8	3.5	Serum Filter
Forage	TBA	1:50	8	3.5	Serum Filter
Seed	TBA	1:50	8	3.5	Serum Filter

[1]Over-season leaf (OSL-1, OSL-2, OSL-3, and OSL-4).

D.5 CP4 EPSPS Antibodies

The capture monoclonal antibody (Lot 10002190) was specific for the CP4 EPSPS protein. The concentration of the purified antibody was determined to be 2.3 mg/ml by spectrophotometric methods. The detection reagent was goat anti-CP4 EPSPS polyclonal antibodies (Sigma, St. Louis, MO) conjugated to horseradish peroxidase (HRP).

D.6 CP4 EPSPS ELISA Method

The CP4 EPSPS ELISA was performed manually according to an in-house SOP. Mouse anti-CP4 EPSPS antibody was diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM NaCl, pH 9.6) and immobilized onto 96-well microtiter plates at 2.0 µg/ml by dispensing 100 µl per well, followed by incubation in a 4°C refrigerator for greater than 12 hours. Plates were washed three times with 1X phosphate buffered saline (PBS) with 0.05% (v:v) Tween-20 (1X PBST) followed by the addition of 100 µl per well of CP4 EPSPS protein standard or sample extract and incubated at 37 °C for one hour. Plates were washed three times with 1X PBST, followed by the addition of 100 µl per well of goat anti-CP4 EPSPS peroxidase conjugate and incubated at 37 °C for one hour. Plates were washed 3 times with 1X PBST, and developed by adding 100 µl per well of HRP substrate, 3,3',5,5'- tetramethylbenzidine (TMB, Kirkegaard & Perry,

Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4 after a 10 minute development. Quantitation of CP4 EPSPS protein was accomplished by interpolation from a CP4 EPSPS protein standard curve that ranged in concentration from 0.456 – 14.6 ng/ml.

D.7 Moisture Analysis

All tissues were analyzed for moisture content using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). 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 in this study. The average 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:

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

The protein levels that were reported to be less than or equal to the LOD or less than the LOQ on a fresh weight basis were not reported on a dry weight basis.

D.8 Data Analyses

The CP4 EPSPS ELISA plates were analyzed on a SPECTRAmax Plus (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 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GxP version 5.0.1 software. 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 converted to 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. Microsoft Excel 2007 (12.0.6324.5001) SP1 MSO (12.0.6320.5000) (Microsoft, Redmond, WA) was used to calculate the CP4 EPSPS protein levels in soybean tissues.

Appendix E. Materials and Methods Used for Compositional Analysis of MON 87705 Soybean Seed, Forage and Processed Fractions

E.1 Materials

MON 87705, a conventional soybean control (A3525), and conventional commercial reference soybean varieties were grown at five locations in Chile in the 2007-2008 growing season. MON 87705 and the conventional control were grown from seed lots GLP-0702-18254-S and GLP-0702-18252-S, respectively. The conventional control material, A3525, has background genetics representative of MON 87705 but does not contain *FATB1-A* and *FAD2-1A* gene segments. Samples from all three replicates of test, control, and reference plots were analyzed with the exception of a single replicate of the control substance from site QUI, which was lost. In addition, 20 commercial conventional soybean varieties were produced alongside of MON 87705 and 19 of these were analyzed for generation of a 99% tolerance interval. One commercial soybean variety had all replicates damaged by an early frost and was excluded from the study (see Section E.3 below). The 19 varieties, locations, and seed lot numbers are listed below:

Material Name	Seed Lot Number	Site Code
Asgrow A2869	GLP-0707-18806-S	SFR
Stine 2788	GLP-0707-18832-S	SFR
Asgrow A3244	GLP-0707-18807-S	SFR
Hoegemeyer 333	GLP-0707-18823-S	SFR
NK 32Z3	GLP-0707-18827-S	CdT
Stine 3300-0	GLP-0707-18833-S	CdT
Channel Bio 3461	GLP-0707-18816-S	CdT
Stewart 3454	GLP-0707-18831-S	CdT
Croplan 3596STS	GLP-0707-18818-S	MEL
Garst 3585N	GLP-0707-18820-S	MEL
Pioneer 93B52	GLP-0707-18829-S	MEL
Quality Plus 365C	GLP-0707-18830-S	MEL
Stine 3600-0	GLP-0707-18834-S	QUI
Channel Bio 37002	GLP-0707-18817-S	QUI
Lewis 372	GLP-0707-18825-S	QUI
Pioneer 93B82	GLP-0707-18828-S	QUI
Lewis 391	GLP-0707-18826-S	RAN
Stine 3870-0	GLP-0707-18835-S	RAN
Asgrow A4324	GLP-0707-18809-S	RAN

E.2. Characterization of the Materials

The identities of the forage and seed samples from MON 87705, conventional 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 seed collected from the field plots. The presence or absence of *FATB1-A* and *FAD2-1A* gene segments in the seed of MON 87705, conventional control and reference soybean varieties was verified by event-specific polymerase chain reaction (PCR). As expected, the results confirm that MON 87705 contains these gene segments and the conventional control and reference varieties do not.

E.3. Field Production of the Samples

A total of 20 different conventional commercial soybean reference varieties were planted at five field locations with four different varieties grown at each site. Seeds were planted in a randomized complete block design with three replicates per block for each test, control, and reference variety. Samples from all three replicates of test, control, and reference plots were analyzed, with the exception of a single replicate of the control variety from site QUI, which was lost in transit from the field location to its destination in the U.S. All replicates of the reference variety UA 4805 from site RAN were damaged by an early frost and were excluded from this study. All the samples were grown under normal agronomic field conditions for their respective geographic regions.

E.4. Summary of Analytical Methods

Harvested soybean seed and forage samples from MON 87705, the conventional control and conventional reference soybean varieties were shipped on dry ice to Covance Laboratories Inc. (Madison, WI) for compositional analyses. Analyses were performed using methods that are currently used to evaluate the nutritional quality of food and feed.

E.4.1. Acid Detergent Fiber

The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected on the frit and determined gravimetrically. The limit of quantitation was 0.100%.

Literature Reference

USDA. 1970. Forage and Fiber Analyses. Agriculture Handbook No. 379. United States Department of Agriculture, Washington, D.C.

E.4.2. Amino Acid Composition

The following 18 amino acids were analyzed:

- Total aspartic acid (including asparagine)
- Total threonine
- Total serine
- Total glutamic acid (including glutamine)
- Total proline
- Total glycine
- Total alanine

- Total valine
- Total isoleucine
- Total leucine
- Total tyrosine
- Total phenylalanine
- Total histidine
- Total lysine
- Total arginine
- Total tryptophan
- Total methionine - (sulfur containing)
- Total cystine (including cysteine) - (sulfur containing)

The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer. The limit of quantitation was 0.100 mg/g.

Reference Standards

- ThermoScientific K18, 2.5 µmol/mL per constituent except cystine (1.25 µmol/mL), Lot Number JC120602
- Sigma, L-Tryptophan, 100%, Lot Number 076K50075
- Sigma/BioChemika, L-Cysteic Acid Monohydrate, >99% (used as 100%), Lot Number 1305674
- Sigma, L-Methionine Sulfone, 100%, Lot Number 012H3349

Literature Reference

AOAC. 2005. Method 982.30 in Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, MD.

E.4.3. Ash

The sample was placed in an electric furnace at 550° C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of quantitation was 0.100%.

Literature Reference

AOAC. 2005. Method 923.03 in Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, MD.

E.4.4. Carbohydrates

The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

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

The limit of quantitation was 0.100%.

Literature Reference
USDA. 1973. Energy value of foods. Agriculture Handbook No. 74. United States Department of Agriculture, Washington, D.C.

E.4.5. Fat by Acid Hydrolysis

The sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted with ether and hexane. The extract was evaporated on a steambath, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated again on a steambath under nitrogen, dried, and weighed. The limit of quantitation was 0.100%.

Literature Reference
AOAC. 2005. Methods 922.06 and 954.02 in Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, MD.

E.4.6. Fat by Soxhlet Extraction

The sample was weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. The limit of quantitation was 0.100%.

Literature Reference
AOAC. 2005. Method 960.39 in Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, MD.

E.4.7. Fatty Acid Profile with Trans Fat by GC

The lipid was extracted, saponified with 0.5 N methanolic sodium hydroxide, and methylated with 14% BF3-methanol. The resulting methyl esters of the fatty acids were extracted with heptane. An internal standard was added prior to the lipid extraction. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The limit of quantitation was 0.0200%.

Reference Standards

- Nu Chek Prep GLC Reference Standard Hazelton No. 1*, Lot Number AU18-S
- Nu Chek Prep GLC Reference Standard Hazelton No. 2*, Lot Number M13-O
- Nu Chek Prep GLC Reference Standard Hazelton No. 3*, Lot Number MA18-S
- Nu Chek Prep GLC Reference Standard Hazelton No. 4*, Lot Number AU18-S
  (*Overall purity of the sum of the mixture of components is 100%)
- Nu Chek Prep Methyl Gamma Linolenate, used as 100%, Lot Number U-63M-JY12-R

- Nu Chek Prep Methyl Tridecanoate, used as 100%, Lot Number N-13M-A2-S
- Nu Chek Prep Methyl Butyrate, used as 100%, Lot Number N-4M-J20-R
- Nu Chek Prep Methyl Hexanoate, used as 100%, Lot Number N-6M-A25-R
- Nu Chek Prep Methyl Erucate, used as 100%, Lot Number U-79M-AU3-Q
- Nu Chek Prep Methyl Lignocerate, used as 100%, Lot Number N-24M-AU18-S
- Nu Chek Prep Methyl Docosapentaenoate, used as 100%, Lot Number U-101M-JY14-S
- Nu Chek Prep Methyl Docosahexaenoate, used as 100%, Lot Number U-84M-M30-S
- Nu Chek Prep Methyl Eicosapentaenoate, used as 100%, Lot Number U-99M-D14-R
- Cayman Chemicals Steariodonic Acid Methyl Ester, used as 100%, Lot Number 182102-1
- Nu Chek Prep Methyl Elaidate, used as 100%, Lot Number U-47M-JA18-R
- Nu Chek Prep Methyl Linoelaidate, used as 100%, Lot Number U-60M-F27-R
- Nu Chek Prep Methyl Nervonate, used as 100%, Lot Number U-88M-AU4-S
- Nu Chek Prep Methyl Palmitelaidate, used as as 100%, Lot Number U-41M-M21-S
- Monsanto Mono Trans SDA, used as 99%, Lot Number GLP-0804-19309-A
- Monsanto Mono Trans Alpha Linolenic Acid, used as 100%, Lot Number GLP-0804-19308-A
- Monsanto 9c, 15c Octadecadienoate (Omnisoy), used as 100%, Lot Number GLP-0802-19168-A
- Larodan Methyl 6(z), 9(z)-Octadecadienoate, used as 99.3%, Lot Number LX-017
- Monsanto Omnisoy C17:1 Methyl 9-cis-Heptadecenoate, used as 99%, Lot Number GLP-0806-19436-A

Literature References

AOAC. 2005. Method 996.06 in Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, MD.

AOCS. 1997. Method Ce 2c-66 and Ce 1C-89 in Official Methods and Recommended Practices of the AOCS, 5th ed. American Oil Chemists' Society, Champaign, IL.

AOAC. 2005. Method 983.23 in Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, MD

E.4.8. Isoflavone Analysis

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

Reference Standards

Chromadex, Daidzein, 96.5%, Lot Number 04007-120

Chromadex, Glycitein, 96.3%, Lot Number 07344-571

Indofine, Genistein, >99%[1], Lot Number 0309074

[1]Used as 100% in calculations.

Literature References

Seo, A. and C.V. Morr. 1984. Improved high-performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. Journal of Agricultural and Food Chemistry, 32(3):530-533.

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

E.4.9. Lectin

The sample was suspended in phosphate buffered saline (PBS), shaken, and filtered. An aliquot of the resulting extract was serially diluted in 10 cuvettes containing PBS. A 10% hematocrit of lyophilized rabbit blood in PBS was added to each dilution. After 2.5 hours, the absorbance of each dilution of the sample and lectin control was measured on a spectrophotometer at 620 nm, using PBS to zero the instrument. One hemagglutinating unit (H.U.) was defined as the level that caused 50% of the standard cell suspension to sediment in 2.5 hours. The limit of quantitation was 0.10 H.U./mg.

Reference Standard

Sigma-Aldrich, Red Blood Cells, Rabbit, Product #R1629, Lot Number 105K6042.

Literature References

Klurfeld, D. M. and Kritchevsky, D. 1987. Isolation and quantitation of lectins from vegetable oils. Lipids 22:667-668.

Klurfeld, D. M., Personal communication.

Liener, I. E., 1955. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. Archives of Biochemistry and Biophysics 54:223-231,

E.4.10. Moisture

The sample was dried in a vacuum oven at approximately 100° C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The limit of quantitation was 0.100%.

Literature Reference

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 926.08 and 925.09, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

E.4.11. Neutral Detergent Fiber, Enzyme Method

The sample was placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected on the frit and determined gravimetrically. The limit of quantitation was 0.100%.

Literature References

Approved Methods of the American Association of Cereal Chemists, 9th Ed., Method 32.20, (1998).

USDA. 1970. Forage and Fiber Analyses. Agriculture Handbook No. 379. United States Department of Agriculture, Washington, D.C.

E.4.12. Phytic Acid

The sample was extracted using 0.5 M HCl with ultrasonication. Purification and concentration were accomplished on a silica-based anion-exchange column. The sample was analyzed on a polymer HPLC column PRP-1, 5 μm (150 x 4.1 mm) with a refractive index detector. The limit of quantitation was 0.100%.

Reference Standard

Aldrich, Phytic Acid, Dodecasodium Salt Hydrate, 95%, Lot Number 077K0693.

Literature References

Lehrfeld, Jacob, "HPLC Separation and Quantitation of Phytic Acid and Some Inositol Phosphates in Foods: Problem and Solutions," Journal of Agricultural and Food Chemistry, 42:2726-2731, (1994).

Lehrfeld, Jacob, "High-Performance Liquid Chromatography Analysis of Phytic Acid on a pH-Stable, Macroporous Polymer Column," Cereal Chemistry, 66(6):510-515, (1989).

E.4.13. Protein

Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25. The limit of quantitation was 0.100%.

Literature References

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 955.04 and 979.09, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

Bradstreet, R. B., The Kjeldahl Method for Organic Nitrogen, Academic Press: New York, New York, (1965).

Kolthoff, I. M., and Sandell, E. B., Quantitative Inorganic Analysis, MacMillan: New York, (1948).

E.4.14. Raffinose and Stachyose

The sample was extracted with deionized water and the extract treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl-β-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography using a flame ionization detector. The quantitation limit was 0.0500%.

Reference Standards

- Sigma, Raffinose Pentahydrate, 99% (84% after correction for degree of hydration), Lot Number 037K1059
- Sigma, Stachyose, 98% (96.8% after correction for degree of hydration), Lot Number 038K3775

Literature References

Brobst, K. M. 1972. Gas-Liquid Chromatography of Trimethylsilyl Derivatives in Methods in Carbohydrate Chemistry, Vol. 6. Academic Press, New York.

Mason, B.S., and H.T. Slover. 1971. A Gas Chromatographic Method for the Determination of Sugars in Foods. Journal of Agricultural and Food Chemistry, 19(3):551-554.

E.4.15. Trypsin Inhibitor

The sample was ground and defatted with petroleum ether. A sample of matrix was extracted with 0.01 N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and benzoy1-DL-arginine~p~nitroanilide hydrochloride. The sample was allowed to react for 10 minutes at 37°C. After 10 minutes, the reaction was halted by the addition of acetic acid. The solution was centrifuged, then the absorbance was determined at 410 nm. Trypsin inhibitor activity was determined by photometrically measuring the inhibition of trypsin's reaction with benzoyl-DL-arginine~p~nitroanilide hydrochloride. The limit of quantitation was 1.00 Trypsin Inhibitor Units (TIU)/mg.

Literature Reference

AOCS. 1997. Method Ba 12-75 in Official Methods and Recommended Practices of the American Oil Chemists' Society. AOCS Press, Champaign, IL.

E.4.16. Vitamin E

The sample was saponified to break down any fat and release vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column. The limit of quantitation was 0.500 mg/100 g.

Reference Standard

USP, Alpha Tocopherol, 100%, Lot Number M.

Literature References

Cort, W.M., T.S. Vincente, E.H. Waysek, and B.D. Williams. 1983. Vitamin E content of feedstuffs determined by high-performance liquid chromatographic Fluorescence. Journal of Agricultural Food Chemistry, 31:1330-1333.

McMurray, C.H., W.J. Blanchflower, and D.A. Rice. 1980. Influence of extraction techniques on determination of α-tocopherol in animal feedstuffs. Journal of the Association of Official Analytical Chemists, 63(6):1258-1261.

Speek, A.J., J. Schijver, and W.H.P. Schreurs. 1985. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric quantitation. Journal of Food Science, 50(1):121-124.

E.5. Data Processing and Statistical Analysis

After compositional analyses were performed at Covance Laboratories, Inc., 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	% FW	% DW	X/d
Amino Acids (AA)	mg/g FW	% DW	X/(10d)
Isoflavones	µg/g FW	µg/g DW	X/d
Trypsin Inhibitor	TIU/mg FW	TIU/mg DW	X/d
Vitamin E	mg/100g FW	mg/100g DW	X/d
Lectin	H.U./mg FW	H.U./mg DW	X/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, at least 50% of the values for a component had to be greater than the assay limit of quantitation (LOQ). Components with more than 50% of observations below the assay LOQ were excluded from summaries and analysis as there was insufficient data to conduct the statistical analysis. The following 17 components with more than 50% of the observations below the assay LOQ were excluded: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecylic acid, 15:1 10c pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 9c heptadecenoic acid, 18:1 9t octadecenoic acid, 18:2 9c,15c octadecadienoic acid, 18:2 6c,9c octadecadienoic acid, 18:3 gamma linolenic acid, 20:2 11c,14c eicosadienoic acid, 20:3 11c,14c,17c eicosatrienoic acid, and 20:4 arachidonic acid.

Otherwise, results below the LOQ were assigned a value equal to one half of the quantitation limit. Thirteen observations for 24:0 lignoceric acid were assigned a value, equal to one half of the quantitation limit (<0.0200%).

PRESS residuals were used to identify outliers. 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 were outside the ±6 studentized PRESS residual range were considered for exclusion from the final analyses. No results had PRESS residual values outside of the ±6 range.

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

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.

For each compositional component, 99% tolerance intervals were calculated. 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. The calculated tolerance intervals in this study are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional soybean. Each tolerance interval estimate was based upon the average of three observations 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.

Table E-1. Statistical Summary of Site CdT Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	32.08 (3.03) [30.53 - 34.11]	27.25 (3.03) [19.21 - 33.03]	4.83 (3.78) [1.02 - 12.39]	-11.44, 21.09	0.329	(23.18 - 42.11) [18.29, 41.02]
Ash	8.74 (0.36) [8.40 - 9.19]	8.53 (0.36) [7.76 - 9.32]	0.22 (0.51) [-0.68 - 1.43]	-1.97, 2.40	0.710	(6.76 - 10.40) [6.78, 9.91]
Carbohydrates	72.60 (0.75) [72.28 - 72.79]	73.03 (0.75) [70.97 - 74.33]	-0.43 (1.05) [-1.61 - 1.82]	-4.96, 4.11	0.724	(63.74 - 80.60) [64.45, 80.50]
Moisture (% FW)	71.53 (0.58) [70.60 - 72.50]	71.17 (0.58) [70.00 - 72.10]	0.37 (0.83) [-1.50 - 2.50]	-3.19, 3.92	0.700	(65.80 - 82.00) [62.26, 83.45]
Neutral Detergent Fiber	37.26 (1.43) [34.35 - 41.05]	32.98 (1.43) [32.26 - 33.67]	4.28 (1.89) [2.10 - 8.05]	-3.86, 12.42	0.151	(24.70 - 46.55) [22.57, 46.52]
Protein	13.34 (0.51) [13.05 - 13.89]	12.97 (0.51) [11.85 - 14.16]	0.37 (0.72) [-1.10 - 2.04]	-2.74, 3.47	0.662	(9.51 - 19.93) [7.38, 21.27]

Table E-1. Statistical Summary of Site CdT Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (%DW)						
Total Fat	5.32 (0.47) [4.77 - 5.71]	5.52 (0.47) [4.40 - 6.47]	-0.20 (0.67) [-1.70 - 1.31]	-3.07, 2.66	0.788	(1.19 - 8.22) [0, 9.74]

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

Table E-2. Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Alanine	1.53 (0.017) [1.51 - 1.54]	1.56 (0.017) [1.51 - 1.58]	-0.023 (0.012) [-0.041 - 0.00038]	-0.077, 0.030	0.200	(1.34 - 1.78) [1.25, 1.92]
Arginine	2.55 (0.036) [2.48 - 2.63]	2.55 (0.036) [2.51 - 2.58]	-0.0053 (0.040) [-0.062 - 0.071]	-0.18, 0.16	0.905	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	3.84 (0.045) [3.76 - 3.93]	3.94 (0.045) [3.86 - 4.00]	-0.10 (0.035) [-0.16 - -0.039]	-0.25, 0.051	0.104	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.62 (0.0081) [0.60 - 0.63]	0.62 (0.0081) [0.60 - 0.63]	0.0014 (0.011) [-0.027 - 0.028]	-0.048, 0.051	0.911	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	5.99 (0.083) [5.86 - 6.13]	6.17 (0.083) [6.00 - 6.29]	-0.19 (0.070) [-0.32 - -0.094]	-0.49, 0.11	0.115	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.52 (0.016) [1.50 - 1.54]	1.55 (0.016) [1.51 - 1.57]	-0.027 (0.0087) [-0.040 - -0.011]	-0.064, 0.010	0.089	(1.30 - 1.79) [1.19, 1.95]

Table E-2 (continued). Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Histidine	0.94 (0.012) [0.92 - 0.97]	0.94 (0.012) [0.92 - 0.96]	0.0024 (0.014) [-0.021 - 0.025]	-0.056, 0.061	0.875	(0.79 - 1.07) [0.74, 1.16]
Isoleucine	1.61 (0.039) [1.57 - 1.64]	1.64 (0.039) [1.55 - 1.71]	-0.034 (0.038) [-0.11 - 0.022]	-0.20, 0.13	0.460	(1.37 - 2.00) [1.23, 2.15]
Leucine	2.56 (0.031) [2.53 - 2.59]	2.63 (0.031) [2.55 - 2.69]	-0.073 (0.030) [-0.13 - -0.026]	-0.20, 0.057	0.137	(2.26 - 3.14) [2.06, 3.41]
Lysine	2.31 (0.026) [2.27 - 2.34]	2.32 (0.026) [2.26 - 2.36]	-0.013 (0.019) [-0.051 - 0.012]	-0.096, 0.071	0.581	(2.00 - 2.63) [1.87, 2.81]
Methionine	0.53 (0.0091) [0.51 - 0.54]	0.54 (0.0091) [0.52 - 0.55]	-0.0089 (0.013) [-0.035 - 0.024]	-0.064, 0.046	0.560	(0.46 - 0.59) [0.43, 0.63]
Phenylalanine	1.71 (0.024) [1.68 - 1.74]	1.76 (0.024) [1.70 - 1.80]	-0.050 (0.024) [-0.097 - -0.025]	-0.15, 0.053	0.172	(1.50 - 2.11) [1.35, 2.31]

Table E-2 (continued). Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Proline	1.65 (0.026) [1.60 - 1.70]	1.68 (0.026) [1.65 - 1.73]	-0.027 (0.011) [-0.046 - -0.0075]	-0.075, 0.021	0.135	(1.43 - 2.03) [1.29, 2.21]
Serine	1.69 (0.069) [1.59 - 1.80]	1.71 (0.069) [1.56 - 1.81]	-0.013 (0.097) [-0.22 - 0.24]	-0.43, 0.40	0.905	(1.55 - 2.05) [1.44, 2.15]
Threonine	1.28 (0.034) [1.25 - 1.34]	1.33 (0.034) [1.26 - 1.39]	-0.050 (0.048) [-0.13 - 0.080]	-0.26, 0.16	0.406	(1.19 - 1.48) [1.12, 1.53]
Tryptophan	0.40 (0.0088) [0.38 - 0.41]	0.42 (0.0088) [0.41 - 0.43]	-0.017 (0.0061) [-0.028 - -0.0070]	-0.043, 0.0094	0.109	(0.33 - 0.48) [0.30, 0.50]
Tyrosine	1.22 (0.021) [1.18 - 1.27]	1.17 (0.021) [1.15 - 1.19]	0.048 (0.029) [-0.0086 - 0.12]	-0.079, 0.17	0.247	(1.07 - 1.39) [0.99, 1.49]
Valine	1.72 (0.042) [1.69 - 1.75]	1.74 (0.042) [1.63 - 1.82]	-0.023 (0.049) [-0.11 - 0.065]	-0.24, 0.19	0.684	(1.45 - 2.13) [1.31, 2.29]

Table E-2 (continued). Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
16:0 Palmitic	2.31 (0.040) [2.29 - 2.32]	10.80 (0.040) [10.72 - 10.91]	-8.49 (0.047) [-8.59 - -8.43]	-8.70, -8.29	<0.001	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic	3.17 (0.040) [3.09 - 3.23]	4.58 (0.040) [4.53 - 4.66]	-1.42 (0.038) [-1.47 - -1.34]	-1.58, -1.25	<0.001	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic	76.44 (0.19) [76.35 - 76.60]	23.02 (0.19) [22.51 - 23.37]	53.42 (0.21) [53.19 - 53.85]	52.50, 54.34	<0.001	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic	10.09 (0.15) [9.94 - 10.22]	52.43 (0.15) [52.13 - 52.78]	-42.34 (0.11) [-42.56 - -42.19]	-42.83, -41.84	<0.001	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic	6.90 (0.047) [6.85 - 6.94]	8.15 (0.047) [8.08 - 8.28]	-1.25 (0.044) [-1.33 - -1.18]	-1.44, -1.06	0.001	(5.89 - 9.11) [4.97, 9.93]
20:0 Arachidic	0.29 (0.0058) [0.28 - 0.29]	0.35 (0.0058) [0.34 - 0.36]	-0.063 (0.0082) [-0.082 - -0.046]	-0.099, -0.028	0.016	(0.28 - 0.54) [0.22, 0.53]

Table E-2 (continued). Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic	0.36 (0.0056) [0.36 - 0.38]	0.21 (0.0056) [0.19 - 0.21]	0.16 (0.0045) [0.15 - 0.16]	0.14, 0.18	<0.001	(0.15 - 0.22) [0.13, 0.25]
22:0 Behenic	0.28 (0.0046) [0.28 - 0.29]	0.30 (0.0046) [0.29 - 0.31]	-0.016 (0.0066) [-0.029 - -0.0044]	-0.045, 0.012	0.131	(0.29 - 0.46) [0.22, 0.47]
24:0 Lignoceric	0.15 (0.0029) [0.14 - 0.15]	0.15 (0.0029) [0.15 - 0.16]	-0.0049 (0.00046) [-0.0055 - -0.0040]	-0.0069, -0.0030	0.008	(0.056 - 0.21) [0.030, 0.26]
Fiber (% DW)						
Acid Detergent Fiber	18.23 (0.32) [17.57 - 18.58]	16.27 (0.32) [15.69 - 16.74]	1.97 (0.45) [0.83 - 2.89]	0.014, 3.92	0.049	(12.46 - 21.25) [12.71, 19.29]
Neutral Detergent Fiber	21.04 (0.45) [20.47 - 22.18]	17.99 (0.45) [17.71 - 18.54]	3.05 (0.30) [2.75 - 3.65]	1.78, 4.33	0.009	(12.25 - 20.89) [12.07, 21.51]
Proximate (% DW)						
Ash	6.37 (0.093) [6.13 - 6.54]	6.22 (0.093) [6.18 - 6.31]	0.15 (0.099) [-0.050 - 0.26]	-0.28, 0.57	0.274	(5.64 - 6.82) [5.26, 7.17]

Table E-2 (continued). Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Proximate (% DW)						
Carbohydrates	41.82 (0.20) [41.62 - 42.00]	40.05 (0.20) [39.64 - 40.52]	1.76 (0.23) [1.31 - 2.00]	0.78, 2.75	0.016	(32.79 - 42.29) [30.78, 45.86]
Moisture (% FW)	10.80 (0.35) [10.10 - 11.20]	10.87 (0.35) [10.20 - 11.40]	-0.067 (0.50) [-1.30 - 0.90]	-2.21, 2.08	0.905	(6.89 - 12.50) [5.51, 13.37]
Protein	33.75 (0.33) [33.26 - 34.65]	34.22 (0.33) [34.04 - 34.41]	-0.47 (0.36) [-0.94 - 0.24]	-2.02, 1.08	0.320	(29.51 - 40.25) [26.12, 43.51]
Total Fat	18.09 (0.25) [17.66 - 18.35]	19.52 (0.25) [18.96 - 19.82]	-1.43 (0.35) [-2.16 - -0.61]	-2.95, 0.083	0.055	(16.91 - 23.48) [15.35, 25.95]
Vitamin (mg/100g DW)						
Vitamin E	3.34 (0.13) [3.17 - 3.50]	4.03 (0.13) [3.74 - 4.19]	-0.69 (0.18) [-1.01 - -0.39]	-1.46, 0.068	0.059	(1.09 - 5.10) [0, 7.36]
Antinutrient						
Lectin (H.U./mg DW)	1.93 (0.74) [1.01 - 2.77]	3.12 (0.74) [2.20 - 4.93]	-1.19 (1.01) [-2.93 - 0.57]	-5.54, 3.15	0.359	(0.65 - 8.10) [0, 6.44]

Table E-2 (continued). Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Antinutrient (% DW)						
Phytic Acid	1.81 (0.10) [1.52 - 1.97]	1.79 (0.10) [1.78 - 1.81]	0.017 (0.14) [-0.26 - 0.16]	-0.59, 0.62	0.914	(1.42 - 2.27) [1.35, 2.35]
Raffinose	0.51 (0.018) [0.50 - 0.54]	0.52 (0.018) [0.50 - 0.56]	-0.0095 (0.025) [-0.069 - 0.030]	-0.12, 0.099	0.741	(0.40 - 0.80) [0.27, 0.87]
Stachyose	3.76 (0.15) [3.55 - 4.16]	3.10 (0.15) [3.04 - 3.21]	0.66 (0.15) [0.51 - 0.95]	0.027, 1.29	0.046	(2.30 - 4.53) [1.96, 4.41]
Trypsin Inhibitor (TIU/mg DW)	42.86 (3.21) [38.51 - 48.03]	43.65 (3.21) [37.36 - 49.78]	-0.79 (2.92) [-5.31 - 4.69]	-13.37, 11.80	0.812	(23.11 - 60.42) [8.75, 63.43]
Isoflavone (µg/g DW)						
Daidzein	1192.24 (52.19) [1145.72 - 1259.84]	1182.58 (52.19) [1092.43 - 1309.26]	9.66 (73.81) [-163.54 - 167.41]	-307.91, 327.23	0.907	(320.54 - 3061.22) [0, 3328.03]
Genistein	813.17 (33.64) [809.79 - 815.32]	825.33 (33.64) [751.67 - 914.22]	-12.17 (47.57) [-104.43 - 62.73]	-216.84, 192.50	0.822	(433.41 - 2301.59) [0, 2727.33]

Table E-2 (continued). Statistical Summary of Site CdT Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Isoflavone (µg/g DW)						
Glycitein	140.40 (18.28) [90.89 - 176.80]	146.58 (18.28) [141.57 - 152.56]	-6.18 (25.85) [-61.67 - 35.23]	-117.41, 105.05	0.833	(21.67 - 354.30) [0, 376.03]

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

Table E-3. Statistical Summary of Site MEL Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	27.77 (1.88) [24.18 - 29.83]	27.46 (1.88) [25.10 - 31.32]	0.31 (1.52) [-1.49 - 3.34]	-6.24, 6.86	0.858	(23.18 - 42.11) [18.29, 41.02]
Ash	8.58 (0.33) [8.32 - 8.86]	8.20 (0.33) [7.66 - 9.08]	0.38 (0.46) [-0.52 - 1.01]	-1.61, 2.38	0.497	(6.76 - 10.40) [6.78, 9.91]
Carbohydrates	71.69 (1.41) [69.52 - 73.15]	71.00 (1.41) [67.88 - 73.56]	0.69 (2.00) [-2.05 - 5.27]	-7.91, 9.28	0.764	(63.74 - 80.60) [64.45, 80.50]
Moisture (% FW)	70.43 (0.26) [70.20 - 70.80]	69.90 (0.26) [69.40 - 70.50]	0.53 (0.37) [-0.20 - 1.40]	-1.06, 2.13	0.287	(65.80 - 82.00) [62.26, 83.45]
Neutral Detergent Fiber	30.89 (1.70) [27.64 - 35.57]	32.58 (1.70) [32.19 - 33.01]	-1.69 (2.21) [-4.90 - 2.56]	-11.22, 7.84	0.524	(24.70 - 46.55) [22.57, 46.52]
Protein	13.95 (1.60) [12.65 - 16.40]	14.11 (1.60) [11.63 - 17.85]	-0.16 (2.27) [-5.20 - 3.56]	-9.90, 9.59	0.951	(9.51 - 19.93) [7.38, 21.27]

Table E-3 (continued). Statistical Summary of Site MEL Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Total Fat	5.79 (0.31) [5.37 - 6.57]	6.76 (0.31) [6.49 - 7.19]	-0.97 (0.17) [-1.16 - -0.62]	-1.71, -0.22	0.030	(1.19 - 8.22) [0, 9.74]

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

Table E-4. Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Alanine	1.62 (0.032) [1.54 - 1.68]	1.56 (0.032) [1.54 - 1.61]	0.052 (0.040) [-0.00088 - 0.13]	-0.12, 0.23	0.323	(1.34 - 1.78) [1.25, 1.92]
Arginine	2.78 (0.097) [2.57 - 2.94]	2.63 (0.097) [2.54 - 2.79]	0.15 (0.12) [0.025 - 0.39]	-0.37, 0.66	0.341	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	4.14 (0.12) [3.87 - 4.36]	3.94 (0.12) [3.84 - 4.15]	0.19 (0.16) [0.026 - 0.52]	-0.51, 0.90	0.354	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.61 (0.013) [0.60 - 0.62]	0.58 (0.013) [0.55 - 0.61]	0.030 (0.018) [-0.0014 - 0.062]	-0.048, 0.11	0.239	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	6.59 (0.23) [6.09 - 7.02]	6.18 (0.23) [5.99 - 6.55]	0.41 (0.31) [0.098 - 1.02]	-0.90, 1.73	0.308	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.61 (0.042) [1.52 - 1.69]	1.55 (0.042) [1.52 - 1.62]	0.060 (0.053) [-0.00070 - 0.17]	-0.17, 0.29	0.374	(1.30 - 1.79) [1.19, 1.95]

Table E-4 (continued). Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Histidine	0.96 (0.025) [0.91 - 1.01]	0.91 (0.025) [0.89 - 0.95]	0.047 (0.033) [0.011 - 0.11]	-0.096, 0.19	0.295	(0.79 - 1.07) [0.74, 1.16]
Isoleucine	1.74 (0.052) [1.61 - 1.84]	1.62 (0.052) [1.58 - 1.66]	0.12 (0.073) [-0.0014 - 0.26]	-0.19, 0.44	0.232	(1.37 - 2.00) [1.23, 2.15]
Leucine	2.78 (0.072) [2.62 - 2.92]	2.65 (0.072) [2.60 - 2.76]	0.13 (0.096) [0.024 - 0.32]	-0.28, 0.54	0.308	(2.26 - 3.14) [2.06, 3.41]
Lysine	2.41 (0.049) [2.31 - 2.50]	2.31 (0.049) [2.25 - 2.39]	0.10 (0.069) [0.016 - 0.25]	-0.19, 0.40	0.278	(2.00 - 2.63) [1.87, 2.81]
Methionine	0.56 (0.0097) [0.53 - 0.57]	0.52 (0.0097) [0.51 - 0.53]	0.038 (0.013) [0.013 - 0.056]	-0.018, 0.094	0.100	(0.46 - 0.59) [0.43, 0.63]
Phenylalanine	1.85 (0.053) [1.74 - 1.95]	1.78 (0.053) [1.73 - 1.87]	0.070 (0.074) [-0.0084 - 0.22]	-0.25, 0.39	0.445	(1.50 - 2.11) [1.35, 2.31]

Table E-4 (continued). Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Proline	1.81 (0.056) [1.69 - 1.89]	1.68 (0.056) [1.63 - 1.78]	0.13 (0.070) [0.043 - 0.27]	-0.18, 0.43	0.213	(1.43 - 2.03) [1.29, 2.21]
Serine	1.79 (0.046) [1.72 - 1.86]	1.79 (0.046) [1.73 - 1.89]	-0.0013 (0.065) [-0.17 - 0.12]	-0.28, 0.28	0.985	(1.55 - 2.05) [1.44, 2.15]
Threonine	1.34 (0.042) [1.31 - 1.39]	1.37 (0.042) [1.28 - 1.47]	-0.025 (0.053) [-0.13 - 0.031]	-0.25, 0.20	0.676	(1.19 - 1.48) [1.12, 1.53]
Tryptophan	0.41 (0.0088) [0.39 - 0.43]	0.41 (0.0088) [0.40 - 0.42]	-0.0023 (0.012) [-0.028 - 0.019]	-0.056, 0.051	0.870	(0.33 - 0.48) [0.30, 0.50]
Tyrosine	1.25 (0.023) [1.23 - 1.26]	1.24 (0.023) [1.20 - 1.30]	0.0014 (0.027) [-0.040 - 0.054]	-0.12, 0.12	0.963	(1.07 - 1.39) [0.99, 1.49]
Valine	1.86 (0.054) [1.72 - 1.96]	1.73 (0.054) [1.68 - 1.76]	0.13 (0.077) [-0.014 - 0.28]	-0.20, 0.46	0.221	(1.45 - 2.13) [1.31, 2.29]

Table E-4 (continued). Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
16:0 Palmitic	2.39 (0.021) [2.35 - 2.42]	10.83 (0.021) [10.80 - 10.87]	-8.44 (0.029) [-8.47 - -8.38]	-8.56, -8.31	<0.001	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic	3.33 (0.10) [3.20 - 3.47]	4.39 (0.10) [4.26 - 4.64]	-1.06 (0.15) [-1.32 - -0.79]	-1.69, -0.42	0.018	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic	76.10 (0.22) [75.68 - 76.33]	22.31 (0.22) [21.86 - 22.61]	53.79 (0.31) [53.06 - 54.47]	52.45, 55.14	<0.001	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic	10.50 (0.22) [10.16 - 10.92]	53.48 (0.22) [53.22 - 53.89]	-42.98 (0.31) [-43.74 - -42.40]	-44.31, -41.66	<0.001	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic	6.58 (0.055) [6.53 - 6.65]	8.00 (0.055) [7.86 - 8.10]	-1.42 (0.076) [-1.56 - -1.31]	-1.74, -1.09	0.002	(5.89 - 9.11) [4.97, 9.93]
20:0 Arachidic	0.30 (0.0058) [0.29 - 0.30]	0.34 (0.0058) [0.32 - 0.34]	-0.040 (0.0066) [-0.052 - -0.030]	-0.068, -0.012	0.026	(0.28 - 0.54) [0.22, 0.53]

Table E-4 (continued). Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic	0.35 (0.0050) [0.34 - 0.36]	0.20 (0.0050) [0.20 - 0.21]	0.14 (0.0063) [0.14 - 0.16]	0.12, 0.17	0.001	(0.15 - 0.22) [0.13, 0.25]
22:0 Behenic	0.30 (0.0063) [0.29 - 0.30]	0.30 (0.0063) [0.28 - 0.31]	0.0014 (0.0089) [-0.016 - 0.020]	-0.037, 0.040	0.891	(0.29 - 0.46) [0.22, 0.47]
24:0 Lignoceric	0.15 (0.0080) [0.14 - 0.17]	0.16 (0.0080) [0.15 - 0.16]	-0.0026 (0.0096) [-0.019 - 0.014]	-0.044, 0.039	0.812	(0.056 - 0.21) [0.030, 0.26]
Fiber (% DW)						
Acid Detergent Fiber	16.35 (0.29) [16.22 - 16.63]	16.74 (0.29) [16.03 - 17.32]	-0.38 (0.29) [-0.69 - 0.19]	-1.61, 0.85	0.313	(12.46 - 21.25) [12.71, 19.29]
Neutral Detergent Fiber	20.24 (0.63) [18.92 - 21.65]	17.83 (0.63) [17.32 - 18.62]	2.42 (0.89) [0.30 - 4.33]	-1.40, 6.23	0.112	(12.25 - 20.89) [12.07, 21.51]
Proximate (% DW)						
Ash	6.11 (0.11) [5.99 - 6.26]	6.41 (0.11) [6.14 - 6.55]	-0.30 (0.14) [-0.56 - -0.073]	-0.90, 0.30	0.164	(5.64 - 6.82) [5.26, 7.17]

Table E-4 (continued). Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Proximate (% DW)						
Carbohydrates	39.70 (0.55) [38.85 - 41.07]	39.60 (0.55) [38.91 - 40.04]	0.10 (0.59) [-0.99 - 1.03]	-2.43, 2.64	0.876	(32.79 - 42.29) [30.78, 45.86]
Moisture (% FW)	10.93 (0.21) [10.40 - 11.20]	11.37 (0.21) [11.10 - 11.60]	-0.43 (0.15) [-0.70 - -0.20]	-1.06, 0.19	0.096	(6.89 - 12.50) [5.51, 13.37]
Protein	35.56 (0.92) [33.59 - 36.94]	34.38 (0.92) [33.30 - 35.97]	1.18 (0.95) [0.18 - 3.08]	-2.89, 5.26	0.337	(29.51 - 40.25) [26.12, 43.51]
Total Fat	18.64 (0.29) [18.24 - 19.08]	19.63 (0.29) [19.00 - 20.13]	-0.99 (0.31) [-1.51 - -0.42]	-2.35, 0.36	0.087	(16.91 - 23.48) [15.35, 25.95]
Vitamin (mg/100g DW)						
Vitamin E	3.26 (0.11) [3.15 - 3.45]	3.83 (0.11) [3.66 - 4.05]	-0.58 (0.043) [-0.64 - -0.49]	-0.76, -0.39	0.005	(1.09 - 5.10) [0, 7.36]
Antinutrient						
Lectin (H.U./mg DW)	1.84 (0.30) [1.61 - 2.14]	1.69 (0.30) [1.04 - 2.40]	0.15 (0.42) [-0.63 - 1.11]	-1.68, 1.98	0.757	(0.65 - 8.10) [0, 6.44]

Table E-4 (continued). Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Antinutrient (% DW)						
Phytic Acid	1.89 (0.099) [1.80 - 2.02]	1.90 (0.099) [1.66 - 2.08]	-0.0094 (0.14) [-0.27 - 0.18]	-0.60, 0.58	0.951	(1.42 - 2.27) [1.35, 2.35]
Raffinose	0.57 (0.019) [0.54 - 0.61]	0.56 (0.019) [0.53 - 0.58]	0.018 (0.0085) [0.0046 - 0.034]	-0.019, 0.054	0.175	(0.40 - 0.80) [0.27, 0.87]
Stachyose	3.68 (0.13) [3.56 - 3.77]	3.78 (0.13) [3.51 - 4.08]	-0.10 (0.18) [-0.53 - 0.26]	-0.87, 0.66	0.615	(2.30 - 4.53) [1.96, 4.41]
Trypsin Inhibitor (TIU/mg DW)	35.12 (2.90) [31.64 - 41.07]	36.06 (2.90) [31.67 - 41.28]	-0.93 (1.36) [-3.57 - 0.98]	-6.80, 4.93	0.564	(23.11 - 60.42) [8.75, 63.43]
Isoflavone (µg/g DW)						
Daidzein	2095.83 (90.09) [2060.81 - 2139.64]	2012.16 (90.09) [1844.77 - 2257.34]	83.67 (127.41) [-196.53 - 242.28]	-464.54, 631.88	0.578	(320.54 - 3061.22) [0, 3328.03]
Genistein	1362.28 (58.14) [1340.09 - 1385.14]	1286.17 (58.14) [1176.47 - 1444.70]	76.11 (82.22) [-104.61 - 208.66]	-277.67, 429.88	0.452	(433.41 - 2301.59) [0, 2727.33]

Table E-4 (continued). Statistical Summary of Site MEL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Isoflavone (µg/g DW)						
Glycitein	144.06 (9.70) [132.88 - 151.79]	142.17 (9.70) [128.23 - 167.04]	1.90 (13.72) [-34.16 - 23.55]	-57.13, 60.93	0.902	(21.67 - 354.30) [0, 376.03]

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

Table E-5. Statistical Summary of Site QUI Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	24.62 (1.87) [20.04 - 26.98]	22.74 (2.29) [22.72 - 22.76]	1.88 (2.96) [4.10 - 4.25]	-35.75, 39.51	0.639	(23.18 - 42.11) [18.29, 41.02]
Ash	8.26 (0.32) [8.02 - 8.51]	7.87 (0.39) [7.24 - 8.49]	0.39 (0.50) [-0.24 - 1.27]	-5.97, 6.76	0.575	(6.76 - 10.40) [6.78, 9.91]
Carbohydrates	71.07 (0.43) [70.11 - 71.67]	72.76 (0.53) [72.40 - 73.12]	-1.69 (0.68) [-3.01 - -0.73]	-10.36, 6.98	0.244	(63.74 - 80.60) [64.45, 80.50]
Moisture (% FW)	71.77 (0.47) [70.70 - 72.70]	72.10 (0.58) [72.10 - 72.10]	-0.33 (0.75) [-1.40 - -0.20]	-9.87, 9.20	0.733	(65.80 - 82.00) [62.26, 83.45]
Neutral Detergent Fiber	30.94 (2.43) [26.12 - 33.59]	32.99 (2.51) [33.91 - 36.56]	-2.05 (1.09) [-2.96 - -0.80]	-15.95, 11.85	0.312	(24.70 - 46.55) [22.57, 46.52]
Protein	16.36 (0.79) [15.22 - 18.10]	15.09 (0.97) [14.37 - 15.81]	1.27 (1.26) [-0.58 - 1.39]	-14.70, 17.24	0.496	(9.51 - 19.93) [7.38, 21.27]

Table E-5 (continued). Statistical Summary of Site QUI Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Total Fat	4.35 (1.10) [2.29 - 5.80]	3.42 (1.15) [3.36 - 5.34]	0.93 (0.57) [0.46 - 1.59]	-6.30, 8.16	0.349	(1.19 - 8.22) [0, 9.74]

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

Table E-6. Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Alanine	1.64 (0.031) [1.60 - 1.70]	1.68 (0.031) [1.63 - 1.66]	-0.036 (0.0029) [-0.039 - -0.033]	-0.072, 0.00038	0.050	(1.34 - 1.78) [1.25, 1.92]
Arginine	2.96 (0.090) [2.82 - 3.12]	2.98 (0.091) [2.82 - 2.99]	-0.022 (0.028) [-0.052 - 0.0034]	-0.38, 0.33	0.577	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	4.31 (0.10) [4.16 - 4.49]	4.40 (0.11) [4.21 - 4.43]	-0.095 (0.053) [-0.16 - -0.051]	-0.77, 0.58	0.323	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.63 (0.0098) [0.60 - 0.64]	0.62 (0.011) [0.61 - 0.63]	0.0059 (0.010) [-0.0061 - 0.017]	-0.12, 0.14	0.666	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	6.85 (0.20) [6.54 - 7.19]	7.06 (0.20) [6.70 - 7.09]	-0.21 (0.058) [-0.27 - -0.16]	-0.94, 0.52	0.171	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.64 (0.029) [1.60 - 1.71]	1.65 (0.035) [1.65 - 1.65]	-0.012 (0.046) [-0.050 - -0.045]	-0.59, 0.57	0.837	(1.30 - 1.79) [1.19, 1.95]

Table E-6 (continued). Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Histidine	0.99 (0.024) [0.95 - 1.04]	1.01 (0.024) [0.97 - 1.01]	-0.018 (0.0035) [-0.021 - -0.014]	-0.062, 0.027	0.124	(0.79 - 1.07) [0.74, 1.16]
Isoleucine	1.76 (0.054) [1.65 - 1.86]	1.87 (0.058) [1.80 - 1.86]	-0.10 (0.036) [-0.15 - -0.074]	-0.56, 0.36	0.214	(1.37 - 2.00) [1.23, 2.15]
Leucine	2.88 (0.073) [2.77 - 3.01]	2.94 (0.074) [2.81 - 2.94]	-0.061 (0.022) [-0.085 - -0.041]	-0.34, 0.22	0.220	(2.26 - 3.14) [2.06, 3.41]
Lysine	2.48 (0.043) [2.41 - 2.55]	2.50 (0.044) [2.42 - 2.51]	-0.019 (0.016) [-0.036 - -0.0050]	-0.22, 0.18	0.438	(2.00 - 2.63) [1.87, 2.81]
Methionine	0.55 (0.0071) [0.54 - 0.57]	0.56 (0.0087) [0.55 - 0.57]	-0.0063 (0.011) [-0.022 - -0.0066]	-0.15, 0.14	0.677	(0.46 - 0.59) [0.43, 0.63]
Phenylalanine	1.89 (0.053) [1.83 - 2.00]	1.93 (0.065) [1.88 - 1.99]	-0.044 (0.084) [-0.16 - -0.035]	-1.11, 1.03	0.695	(1.50 - 2.11) [1.35, 2.31]

Table E-6 (continued). Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Proline	1.85 (0.045) [1.77 - 1.92]	1.88 (0.045) [1.80 - 1.90]	-0.032 (0.0091) [-0.041 - -0.023]	-0.15, 0.083	0.176	(1.43 - 2.03) [1.29, 2.21]
Serine	1.92 (0.051) [1.87 - 1.98]	1.83 (0.062) [1.74 - 1.92]	0.083 (0.080) [-0.053 - 0.16]	-0.93, 1.10	0.486	(1.55 - 2.05) [1.44, 2.15]
Threonine	1.42 (0.023) [1.38 - 1.45]	1.37 (0.028) [1.34 - 1.41]	0.046 (0.037) [-0.025 - 0.11]	-0.42, 0.51	0.429	(1.19 - 1.48) [1.12, 1.53]
Tryptophan	0.43 (0.0092) [0.41 - 0.45]	0.44 (0.011) [0.43 - 0.44]	-0.0041 (0.015) [-0.025 - -0.0021]	-0.19, 0.18	0.826	(0.33 - 0.48) [0.30, 0.50]
Tyrosine	1.31 (0.018) [1.29 - 1.32]	1.28 (0.021) [1.25 - 1.32]	0.024 (0.028) [-0.024 - 0.059]	-0.33, 0.38	0.543	(1.07 - 1.39) [0.99, 1.49]
Valine	1.86 (0.059) [1.73 - 1.96]	1.96 (0.066) [1.90 - 1.96]	-0.10 (0.053) [-0.17 - -0.064]	-0.77, 0.56	0.300	(1.45 - 2.13) [1.31, 2.29]

Table E-6 (continued). Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
16:0 Palmitic	2.30 (0.042) [2.25 - 2.37]	10.56 (0.051) [10.51 - 10.62]	-8.26 (0.066) [-8.37 - -8.13]	-9.10, -7.43	0.005	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic	3.51 (0.19) [3.15 - 3.82]	4.82 (0.19) [4.47 - 4.85]	-1.31 (0.0088) [-1.32 - -1.30]	-1.42, -1.20	0.004	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic	78.61 (0.41) [77.70 - 79.17]	24.95 (0.44) [24.34 - 25.08]	53.66 (0.26) [53.36 - 53.88]	50.35, 56.96	0.003	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic	8.75 (0.58) [7.85 - 10.02]	51.70 (0.63) [51.68 - 52.44]	-42.95 (0.44) [-43.30 - -42.42]	-48.50, -37.39	0.006	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic	5.64 (0.074) [5.55 - 5.71]	7.02 (0.082) [6.86 - 7.16]	-1.38 (0.063) [-1.45 - -1.32]	-2.19, -0.58	0.029	(5.89 - 9.11) [4.97, 9.93]
20:0 Arachidic	0.33 (0.017) [0.30 - 0.36]	0.36 (0.017) [0.33 - 0.36]	-0.032 (0.0021) [-0.034 - -0.030]	-0.058, -0.0055	0.041	(0.28 - 0.54) [0.22, 0.53]

Table E-6 (continued). Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic	0.38 (0.0089) [0.37 - 0.40]	0.20 (0.011) [0.20 - 0.20]	0.18 (0.014) [0.18 - 0.20]	0.0019, 0.36	0.049	(0.15 - 0.22) [0.13, 0.25]
22:0 Behenic	0.31 (0.0094) [0.30 - 0.33]	0.31 (0.0095) [0.29 - 0.31]	0.0051 (0.0029) [0.0019 - 0.0078]	-0.032, 0.042	0.330	(0.29 - 0.46) [0.22, 0.47]
24:0 Lignoceric	0.17 (0.0027) [0.17 - 0.17]	0.15 (0.0033) [0.15 - 0.16]	0.021 (0.0042) [0.015 - 0.027]	-0.032, 0.075	0.124	(0.056 - 0.21) [0.030, 0.26]
Fiber (% DW)						
Acid Detergent Fiber	18.15 (0.59) [17.23 - 19.31]	15.96 (0.73) [15.28 - 16.65]	2.19 (0.94) [1.28 - 4.03]	-9.75, 14.13	0.258	(12.46 - 21.25) [12.71, 19.29]
Neutral Detergent Fiber	18.01 (0.71) [16.93 - 19.69]	16.53 (0.87) [16.18 - 16.87]	1.48 (1.13) [0.054 - 1.23]	-12.86, 15.82	0.414	(12.25 - 20.89) [12.07, 21.51]
Proximate (% DW)						
Ash	6.22 (0.089) [6.06 - 6.42]	6.10 (0.10) [6.13 - 6.18]	0.12 (0.091) [0.061 - 0.24]	-1.04, 1.28	0.414	(5.64 - 6.82) [5.26, 7.17]

Table E-6 (continued). Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Proximate (% DW)						
Carbohydrates	37.54 (0.51) [36.69 - 38.62]	37.41 (0.55) [37.46 - 38.09]	0.12 (0.34) [-0.15 - 0.53]	-4.20, 4.44	0.779	(32.79 - 42.29) [30.78, 45.86]
Moisture (% FW)	10.40 (0.097) [10.20 - 10.60]	11.05 (0.12) [11.00 - 11.10]	-0.65 (0.15) [-0.90 - -0.60]	-2.60, 1.30	0.147	(6.89 - 12.50) [5.51, 13.37]
Protein	37.05 (0.83) [35.60 - 38.59]	37.11 (0.84) [35.96 - 36.78]	-0.055 (0.27) [-0.35 - 0.19]	-3.50, 3.39	0.873	(29.51 - 40.25) [26.12, 43.51]
Total Fat	19.20 (0.21) [18.68 - 19.49]	19.73 (0.26) [19.69 - 19.78]	-0.53 (0.34) [-0.36 - -0.20]	-4.80, 3.73	0.356	(16.91 - 23.48) [15.35, 25.95]
Vitamin (mg/100g DW)						
Vitamin E	3.93 (0.19) [3.70 - 4.36]	3.72 (0.23) [3.61 - 3.82]	0.21 (0.29) [-0.13 - 0.76]	-3.51, 3.93	0.599	(1.09 - 5.10) [0, 7.36]
Antinutrient						
Lectin (H.U./mg DW)	1.45 (0.82) [0.72 - 2.03]	3.92 (1.00) [2.32 - 5.53]	-2.47 (1.30) [-4.80 - -0.29]	-18.93, 13.99	0.307	(0.65 - 8.10) [0, 6.44]

Table E-6 (continued). Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Antinutrient (% DW)						
Phytic Acid	1.89 (0.12) [1.73 - 2.13]	1.97 (0.12) [1.90 - 2.19]	-0.085 (0.024) [-0.11 - -0.059]	-0.39, 0.22	0.178	(1.42 - 2.27) [1.35, 2.35]
Raffinose	0.60 (0.023) [0.56 - 0.64]	0.55 (0.029) [0.52 - 0.57]	0.049 (0.037) [-0.015 - 0.12]	-0.42, 0.52	0.412	(0.40 - 0.80) [0.27, 0.87]
Stachyose	4.08 (0.31) [3.43 - 4.48]	3.84 (0.38) [3.52 - 4.16]	0.24 (0.49) [0.18 - 0.96]	-5.93, 6.42	0.703	(2.30 - 4.53) [1.96, 4.41]
Trypsin Inhibitor (TIU/mg DW)	42.52 (5.28) [33.22 - 52.01]	40.77 (5.31) [41.51 - 49.21]	1.75 (0.99) [0.81 - 2.80]	-10.88, 14.38	0.329	(23.11 - 60.42) [8.75, 63.43]
Isoflavone (µg/g DW)						
Daidzein	1346.63 (67.43) [1230.43 - 1450.89]	1411.91 (69.07) [1394.83 - 1539.33]	-65.28 (26.14) [-88.43 - -36.25]	-397.44, 266.88	0.242	(320.54 - 3061.22) [0, 3328.03]
Genistein	952.69 (44.70) [882.55 - 1005.58]	932.29 (52.06) [881.89 - 1020.22]	20.40 (50.70) [-14.64 - 88.04]	-623.77, 664.56	0.756	(433.41 - 2301.59) [0, 2727.33]

Table E-6 (continued). Statistical Summary of Site QUI Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Isoflavone (µg/g DW)						
Glycitein	87.69 (17.12) [49.11 - 108.04]	98.58 (18.14) [72.10 - 109.21]	-10.89 (10.64) [-22.99 - -1.18]	-146.05, 124.28	0.492	(21.67 - 354.30) [0, 376.03]

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

Table E-7. Statistical Summary of Site RAN Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	32.11 (2.53) [31.02 - 33.39]	29.37 (2.53) [24.37 - 36.13]	2.73 (3.57) [-4.22 - 9.02]	-12.65, 18.11	0.524	(23.18 - 42.11) [18.29, 41.02]
Ash	9.64 (0.30) [8.81 - 10.11]	8.56 (0.30) [8.40 - 8.67]	1.07 (0.42) [0.14 - 1.60]	-0.75, 2.90	0.126	(6.76 - 10.40) [6.78, 9.91]
Carbohydrates	69.77 (0.62) [68.94 - 71.06]	72.09 (0.62) [70.97 - 72.89]	-2.32 (0.40) [-3.10 - -1.83]	-4.02, -0.62	0.027	(63.74 - 80.60) [64.45, 80.50]
Moisture (% FW)	78.03 (1.32) [76.50 - 81.10]	75.53 (1.32) [73.90 - 77.50]	2.50 (1.86) [-1.00 - 7.20]	-5.50, 10.50	0.311	(65.80 - 82.00) [62.26, 83.45]
Neutral Detergent Fiber	41.60 (1.83) [39.19 - 46.30]	36.03 (1.83) [33.91 - 37.55]	5.58 (1.75) [2.70 - 8.75]	-1.97, 13.12	0.086	(24.70 - 46.55) [22.57, 46.52]
Protein	16.73 (0.63) [15.19 - 17.78]	14.71 (0.63) [14.02 - 15.48]	2.02 (0.89) [0.57 - 3.75]	-1.82, 5.87	0.151	(9.51 - 19.93) [7.38, 21.27]

Table E-7 (continued). Statistical Summary of Site RAN Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Total Fat	3.87 (0.48) [2.83 - 4.89]	4.66 (0.48) [4.05 - 5.06]	-0.80 (0.67) [-2.23 - 0.015]	-3.69, 2.10	0.358	(1.19 - 8.22) [0, 9.74]

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

Table E-8. Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Alanine	1.66 (0.021) [1.62 - 1.70]	1.62 (0.021) [1.59 - 1.66]	0.036 (0.027) [-0.0069 - 0.085]	-0.080, 0.15	0.314	(1.34 - 1.78) [1.25, 1.92]
Arginine	3.08 (0.032) [3.01 - 3.16]	2.95 (0.032) [2.94 - 2.97]	0.13 (0.045) [0.043 - 0.22]	-0.062, 0.33	0.099	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	4.35 (0.036) [4.30 - 4.44]	4.24 (0.036) [4.20 - 4.29]	0.11 (0.051) [0.026 - 0.23]	-0.11, 0.33	0.173	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.60 (0.011) [0.57 - 0.62]	0.58 (0.011) [0.57 - 0.59]	0.022 (0.011) [0.00097 - 0.033]	-0.024, 0.069	0.171	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	7.00 (0.060) [6.92 - 7.14]	6.82 (0.060) [6.73 - 6.89]	0.17 (0.085) [0.039 - 0.41]	-0.19, 0.54	0.177	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.68 (0.030) [1.62 - 1.74]	1.63 (0.030) [1.58 - 1.67]	0.046 (0.020) [0.015 - 0.084]	-0.041, 0.13	0.150	(1.30 - 1.79) [1.19, 1.95]

Table E-8 (continued). Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Histidine	1.02 (0.0088) [1.01 - 1.04]	0.99 (0.0088) [0.98 - 1.01]	0.031 (0.012) [0.0086 - 0.060]	-0.022, 0.085	0.127	(0.79 - 1.07) [0.74, 1.16]
Isoleucine	1.83 (0.022) [1.78 - 1.88]	1.76 (0.022) [1.74 - 1.77]	0.069 (0.031) [0.0045 - 0.14]	-0.063, 0.20	0.153	(1.37 - 2.00) [1.23, 2.15]
Leucine	2.91 (0.022) [2.89 - 2.97]	2.86 (0.022) [2.85 - 2.90]	0.049 (0.031) [-0.0094 - 0.12]	-0.085, 0.18	0.256	(2.26 - 3.14) [2.06, 3.41]
Lysine	2.49 (0.021) [2.46 - 2.55]	2.43 (0.021) [2.41 - 2.45]	0.065 (0.030) [0.018 - 0.13]	-0.065, 0.19	0.164	(2.00 - 2.63) [1.87, 2.81]
Methionine	0.57 (0.0092) [0.55 - 0.58]	0.53 (0.0092) [0.52 - 0.54]	0.035 (0.010) [0.015 - 0.049]	-0.0098, 0.079	0.078	(0.46 - 0.59) [0.43, 0.63]
Phenylalanine	1.95 (0.013) [1.93 - 1.98]	1.92 (0.013) [1.90 - 1.94]	0.028 (0.018) [-0.0066 - 0.075]	-0.050, 0.11	0.265	(1.50 - 2.11) [1.35, 2.31]

Table E-8 (continued). Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Proline	1.92 (0.018) [1.88 - 1.95]	1.83 (0.018) [1.80 - 1.85]	0.093 (0.025) [0.026 - 0.14]	-0.014, 0.20	0.064	(1.43 - 2.03) [1.29, 2.21]
Serine	1.79 (0.041) [1.74 - 1.90]	1.91 (0.041) [1.88 - 1.94]	-0.11 (0.058) [-0.20 - 0.0033]	-0.37, 0.14	0.186	(1.55 - 2.05) [1.44, 2.15]
Threonine	1.36 (0.014) [1.33 - 1.39]	1.36 (0.014) [1.34 - 1.38]	-0.00098 (0.020) [-0.052 - 0.042]	-0.088, 0.086	0.965	(1.19 - 1.48) [1.12, 1.53]
Tryptophan	0.45 (0.0040) [0.44 - 0.46]	0.44 (0.0040) [0.44 - 0.44]	0.0065 (0.0057) [-0.0022 - 0.022]	-0.018, 0.031	0.372	(0.33 - 0.48) [0.30, 0.50]
Tyrosine	1.29 (0.014) [1.26 - 1.33]	1.30 (0.014) [1.29 - 1.31]	-0.0067 (0.014) [-0.026 - 0.019]	-0.065, 0.052	0.670	(1.07 - 1.39) [0.99, 1.49]
Valine	1.97 (0.023) [1.91 - 2.02]	1.89 (0.023) [1.87 - 1.91]	0.080 (0.033) [0.026 - 0.15]	-0.063, 0.22	0.137	(1.45 - 2.13) [1.31, 2.29]

Table E-8 (continued). Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
16:0 Palmitic	2.40 (0.0088) [2.39 - 2.40]	10.96 (0.0088) [10.94 - 10.98]	-8.56 (0.012) [-8.58 - -8.54]	-8.61, -8.51	<0.001	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic	3.34 (0.032) [3.28 - 3.41]	4.50 (0.032) [4.47 - 4.55]	-1.16 (0.045) [-1.22 - -1.07]	-1.35, -0.96	0.001	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic	74.69 (0.59) [73.13 - 75.98]	21.53 (0.59) [21.41 - 21.63]	53.15 (0.79) [51.71 - 54.43]	49.76, 56.55	<0.001	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic	11.32 (0.42) [10.37 - 12.42]	53.73 (0.42) [53.67 - 53.81]	-42.41 (0.57) [-43.33 - -41.38]	-44.84, -39.97	<0.001	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic	7.32 (0.19) [6.87 - 7.81]	8.43 (0.19) [8.40 - 8.47]	-1.11 (0.27) [-1.60 - -0.59]	-2.28, 0.066	0.055	(5.89 - 9.11) [4.97, 9.93]
20:0 Arachidic	0.28 (0.0036) [0.28 - 0.29]	0.33 (0.0036) [0.32 - 0.33]	-0.043 (0.0051) [-0.053 - -0.031]	-0.065, -0.021	0.014	(0.28 - 0.54) [0.22, 0.53]

Table E-8 (continued). Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic	0.29 (0.0087) [0.27 - 0.31]	0.16 (0.0087) [0.15 - 0.16]	0.13 (0.0081) [0.12 - 0.15]	0.096, 0.17	0.003	(0.15 - 0.22) [0.13, 0.25]
22:0 Behenic	0.28 (0.0041) [0.28 - 0.29]	0.30 (0.0041) [0.30 - 0.30]	-0.016 (0.0047) [-0.021 - -0.0062]	-0.036, 0.0048	0.080	(0.29 - 0.46) [0.22, 0.47]
24:0 Lignoceric	0.069 (0.0011) [0.066 - 0.071]	0.067 (0.0011) [0.067 - 0.068]	0.0017 (0.0012) [-0.00065 - 0.0032]	-0.0034, 0.0069	0.285	(0.056 - 0.21) [0.030, 0.26]
Fiber(% DW)						
Acid Detergent Fiber	16.32 (0.34) [15.71 - 16.78]	13.94 (0.34) [13.36 - 14.58]	2.38 (0.11) [2.20 - 2.58]	1.91, 2.85	0.002	(12.46 - 21.25) [12.71, 19.29]
Neutral Detergent Fiber	15.14 (0.91) [13.41 - 16.48]	16.40 (0.91) [14.61 - 17.51]	-1.26 (0.39) [-1.97 - -0.61]	-2.95, 0.43	0.085	(12.25 - 20.89) [12.07, 21.51]
Proximate (% DW)						
Ash	5.53 (0.088) [5.46 - 5.63]	5.68 (0.088) [5.48 - 5.88]	-0.14 (0.12) [-0.42 - 0.15]	-0.68, 0.39	0.370	(5.64 - 6.82) [5.26, 7.17]

Table E-8 (continued). Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Proximate (% DW)						
Carbohydrates	40.12 (0.24) [39.56 - 40.64]	39.60 (0.24) [39.41 - 39.84]	0.52 (0.34) [-0.28 - 1.23]	-0.93, 1.97	0.262	(32.79 - 42.29) [30.78, 45.86]
Moisture (% FW)	9.52 (0.43) [8.96 - 10.60]	12.03 (0.43) [11.50 - 12.40]	-2.51 (0.61) [-3.40 - -0.90]	-5.12, 0.090	0.053	(6.89 - 12.50) [5.51, 13.37]
Protein	37.54 (0.20) [37.35 - 37.70]	36.87 (0.20) [36.56 - 37.40]	0.67 (0.19) [0.29 - 0.94]	-0.16, 1.51	0.074	(29.51 - 40.25) [26.12, 43.51]
Total Fat	16.83 (0.21) [16.55 - 17.36]	17.85 (0.21) [17.63 - 18.11]	-1.01 (0.30) [-1.52 - -0.45]	-2.30, 0.27	0.077	(16.91 - 23.48) [15.35, 25.95]
Vitamin (mg/100g DW)						
Vitamin E	1.44 (0.094) [1.23 - 1.64]	1.81 (0.094) [1.69 - 1.91]	-0.38 (0.094) [-0.47 - -0.19]	-0.78, 0.026	0.056	(1.09 - 5.10) [0, 7.36]
Antinutrient						
Lectin (H.U./mg DW)	3.57 (0.50) [3.30 - 3.77]	1.36 (0.50) [0.61 - 2.73]	2.21 (0.61) [1.04 - 3.04]	-0.39, 4.82	0.067	(0.65 - 8.10) [0, 6.44]

Table E-8 (continued). Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Antinutrient (% DW)						
Phytic Acid	1.62 (0.032) [1.61 - 1.63]	1.72 (0.032) [1.63 - 1.77]	-0.10 (0.042) [-0.14 - -0.016]	-0.28, 0.079	0.139	(1.42 - 2.27) [1.35, 2.35]
Raffinose	0.52 (0.017) [0.48 - 0.56]	0.56 (0.017) [0.55 - 0.57]	-0.044 (0.023) [-0.088 - -0.011]	-0.14, 0.056	0.198	(0.40 - 0.80) [0.27, 0.87]
Stachyose	3.79 (0.21) [3.39 - 4.35]	3.70 (0.21) [3.56 - 3.80]	0.084 (0.30) [-0.35 - 0.79]	-1.20, 1.37	0.804	(2.30 - 4.53) [1.96, 4.41]
Trypsin Inhibitor (TIU/mg DW)	29.38 (1.10) [26.73 - 31.10]	28.54 (1.10) [27.23 - 29.95]	0.85 (0.95) [-0.50 - 2.67]	-3.23, 4.92	0.465	(23.11 - 60.42) [8.75, 63.43]
Isoflavone (µg/g DW)						
Daidzein	1915.89 (53.73) [1868.13 - 1946.31]	1947.43 (53.73) [1803.65 - 2027.33]	-31.54 (48.74) [-94.12 - 64.48]	-241.26, 178.18	0.583	(320.54 - 3061.22) [0, 3328.03]
Genistein	1186.26 (34.98) [1164.84 - 1208.26]	1149.45 (34.98) [1054.79 - 1209.04]	36.81 (39.06) [-23.36 - 110.04]	-131.24, 204.87	0.445	(433.41 - 2301.59) [0, 2727.33]

Table E-8 (continued). Statistical Summary of Site RAN Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Isoflavone (µg/g DW)						
Glycitein	117.22 (15.47) [79.19 - 140.66]	130.37 (15.47) [110.84 - 146.92]	-13.15 (21.88) [-54.14 - 29.81]	-107.29, 80.99	0.608	(21.67 - 354.30) [0, 376.03]

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

Table E-9. Statistical Summary of Site SFR Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	34.33 (3.08) [30.23 - 42.14]	38.52 (3.08) [34.68 - 40.67]	-4.19 (4.36) [-10.04 - 7.47]	-22.93, 14.55	0.437	(23.18 - 42.11) [18.29, 41.02]
Ash	8.54 (0.51) [7.39 - 9.74]	7.73 (0.51) [7.21 - 8.02]	0.81 (0.73) [-0.63 - 1.79]	-2.31, 3.94	0.379	(6.76 - 10.40) [6.78, 9.91]
Carbohydrates	76.37 (1.00) [74.24 - 78.93]	78.35 (1.00) [77.70 - 78.99]	-1.98 (1.42) [-4.74 - 1.23]	-8.09, 4.13	0.297	(63.74 - 80.60) [64.45, 80.50]
Moisture (% FW)	72.87 (0.39) [72.00 - 73.60]	72.60 (0.39) [72.20 - 73.20]	0.27 (0.47) [-0.20 - 1.20]	-1.74, 2.27	0.625	(65.80 - 82.00) [62.26, 83.45]
Neutral Detergent Fiber	38.24 (1.13) [37.27 - 38.89]	43.34 (1.13) [40.65 - 45.90]	-5.10 (1.53) [-7.01 - -2.08]	-11.67, 1.48	0.079	(24.70 - 46.55) [22.57, 46.52]
Protein	9.84 (0.24) [9.25 - 10.42]	9.77 (0.24) [9.71 - 9.81]	0.070 (0.32) [-0.46 - 0.63]	-1.29, 1.43	0.845	(9.51 - 19.93) [7.38, 21.27]

Table E-9 (continued). Statistical Summary of Site SFR Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Total Fat	5.25 (0.64) [4.36 - 7.01]	4.31 (0.64) [3.99 - 4.75]	0.93 (0.91) [-0.39 - 2.80]	-2.98, 4.84	0.413	(1.19 - 8.22) [0, 9.74]

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

Table E-10. Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Alanine	1.51 (0.011) [1.49 - 1.54]	1.44 (0.011) [1.43 - 1.45]	0.067 (0.011) [0.055 - 0.088]	0.021, 0.11	0.024	(1.34 - 1.78) [1.25, 1.92]
Arginine	2.52 (0.048) [2.43 - 2.64]	2.34 (0.048) [2.31 - 2.39]	0.18 (0.040) [0.11 - 0.24]	0.0051, 0.35	0.047	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	3.76 (0.054) [3.67 - 3.88]	3.56 (0.054) [3.51 - 3.65]	0.20 (0.019) [0.17 - 0.23]	0.11, 0.28	0.009	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.61 (0.010) [0.60 - 0.62]	0.57 (0.010) [0.55 - 0.59]	0.043 (0.015) [0.0062 - 0.073]	-0.020, 0.11	0.098	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	5.90 (0.11) [5.72 - 6.12]	5.53 (0.11) [5.42 - 5.71]	0.37 (0.034) [0.30 - 0.41]	0.22, 0.51	0.008	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.49 (0.015) [1.47 - 1.54]	1.42 (0.015) [1.41 - 1.43]	0.078 (0.022) [0.055 - 0.12]	-0.016, 0.17	0.069	(1.30 - 1.79) [1.19, 1.95]

Table-10 (continued). Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Histidine	0.90 (0.014) [0.88 - 0.94]	0.85 (0.014) [0.84 - 0.87]	0.050 (0.0069) [0.043 - 0.064]	0.021, 0.080	0.018	(0.79 - 1.07) [0.74, 1.16]
Isoleucine	1.60 (0.029) [1.56 - 1.66]	1.51 (0.029) [1.45 - 1.54]	0.093 (0.030) [0.032 - 0.12]	-0.037, 0.22	0.091	(1.37 - 2.00) [1.23, 2.15]
Leucine	2.54 (0.036) [2.47 - 2.61]	2.41 (0.036) [2.37 - 2.47]	0.12 (0.015) [0.095 - 0.15]	0.059, 0.19	0.014	(2.26 - 3.14) [2.06, 3.41]
Lysine	2.25 (0.026) [2.19 - 2.30]	2.13 (0.026) [2.10 - 2.17]	0.11 (0.010) [0.097 - 0.13]	0.070, 0.16	0.007	(2.00 - 2.63) [1.87, 2.81]
Methionine	0.53 (0.0080) [0.52 - 0.55]	0.50 (0.0080) [0.49 - 0.51]	0.037 (0.011) [0.0074 - 0.056]	-0.011, 0.086	0.081	(0.46 - 0.59) [0.43, 0.63]
Phenylalanine	1.68 (0.023) [1.64 - 1.73]	1.60 (0.023) [1.58 - 1.64]	0.077 (0.011) [0.056 - 0.089]	0.030, 0.12	0.019	(1.50 - 2.11) [1.35, 2.31]

Table E-10 (continued). Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Proline	1.62 (0.023) [1.59 - 1.66]	1.55 (0.023) [1.52 - 1.60]	0.078 (0.012) [0.065 - 0.10]	0.028, 0.13	0.021	(1.43 - 2.03) [1.29, 2.21]
Serine	1.56 (0.063) [1.49 - 1.68]	1.58 (0.063) [1.45 - 1.68]	-0.017 (0.089) [-0.15 - 0.23]	-0.40, 0.36	0.863	(1.55 - 2.05) [1.44, 2.15]
Threonine	1.24 (0.022) [1.20 - 1.28]	1.22 (0.022) [1.18 - 1.25]	0.022 (0.032) [-0.044 - 0.10]	-0.11, 0.16	0.559	(1.19 - 1.48) [1.12, 1.53]
Tryptophan	0.39 (0.011) [0.37 - 0.41]	0.37 (0.011) [0.35 - 0.38]	0.024 (0.016) [-0.0073 - 0.046]	-0.045, 0.092	0.276	(0.33 - 0.48) [0.30, 0.50]
Tyrosine	1.18 (0.0097) [1.17 - 1.20]	1.12 (0.0097) [1.10 - 1.13]	0.064 (0.014) [0.039 - 0.10]	0.0052, 0.12	0.042	(1.07 - 1.39) [0.99, 1.49]
Valine	1.72 (0.031) [1.69 - 1.79]	1.61 (0.031) [1.55 - 1.64]	0.12 (0.036) [0.044 - 0.16]	-0.039, 0.27	0.084	(1.45 - 2.13) [1.31, 2.29]

Table E-10 (continued). Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
16:0 Palmitic	2.42 (0.029) [2.40 - 2.44]	11.00 (0.029) [10.95 - 11.08]	-8.58 (0.042) [-8.68 - -8.53]	-8.76, -8.40	<0.001	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic	3.22 (0.084) [3.07 - 3.41]	4.31 (0.084) [4.24 - 4.44]	-1.09 (0.044) [-1.17 - -1.03]	-1.28, -0.90	0.001	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic	76.49 (0.43) [75.33 - 77.21]	22.42 (0.43) [22.16 - 22.65]	54.07 (0.60) [52.87 - 55.05]	51.47, 56.67	<0.001	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic	9.82 (0.27) [9.33 - 10.55]	52.84 (0.27) [52.75 - 52.98]	-43.02 (0.38) [-43.65 - -42.21]	-44.64, -41.39	<0.001	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic	6.98 (0.11) [6.79 - 7.26]	8.49 (0.11) [8.41 - 8.60]	-1.50 (0.15) [-1.80 - -1.20]	-2.16, -0.85	0.009	(5.89 - 9.11) [4.97, 9.93]
20:0 Arachidic	0.29 (0.0035) [0.29 - 0.29]	0.32 (0.0035) [0.31 - 0.32]	-0.026 (0.0021) [-0.029 - -0.022]	-0.035, -0.017	0.006	(0.28 - 0.54) [0.22, 0.53]

Table E-10 (continued). Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic	0.33 (0.0077) [0.32 - 0.35]	0.18 (0.0077) [0.17 - 0.19]	0.15 (0.011) [0.13 - 0.17]	0.10, 0.19	0.005	(0.15 - 0.22) [0.13, 0.25]
22:0 Behenic	0.29 (0.0025) [0.29 - 0.29]	0.30 (0.0025) [0.29 - 0.30]	-0.0071 (0.0033) [-0.012 - -0.0012]	-0.021, 0.0069	0.161	(0.29 - 0.46) [0.22, 0.47]
24:0 Lignoceric	0.15 (0.0045) [0.14 - 0.16]	0.14 (0.0045) [0.13 - 0.14]	0.0090 (0.0039) [0.0013 - 0.014]	-0.0077, 0.026	0.145	(0.056 - 0.21) [0.030, 0.26]
Fiber (% DW)						
Acid Detergent Fiber	16.62 (0.28) [15.85 - 17.05]	17.78 (0.28) [17.64 - 18.02]	-1.17 (0.36) [-1.84 - -0.59]	-2.74, 0.40	0.085	(12.46 - 21.25) [12.71, 19.29]
Neutral Detergent Fiber	17.75 (0.93) [15.62 - 19.68]	19.99 (0.93) [19.01 - 21.09]	-2.24 (1.01) [-4.24 - -1.06]	-6.57, 2.09	0.156	(12.25 - 20.89) [12.07, 21.51]
Proximate (% DW)						
Ash	6.05 (0.10) [5.84 - 6.32]	6.17 (0.10) [6.15 - 6.22]	-0.12 (0.14) [-0.38 - 0.16]	-0.73, 0.49	0.491	(5.64 - 6.82) [5.26, 7.17]

Table E-10 (continued). Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Proximate (% DW)						
Carbohydrates	42.56 (0.37) [41.73 - 43.52]	42.80 (0.37) [42.65 - 42.96]	-0.24 (0.47) [-1.08 - 0.57]	-2.28, 1.80	0.663	(32.79 - 42.29) [30.78, 45.86]
Moisture (% FW)	12.13 (0.11) [12.00 - 12.30]	12.47 (0.11) [12.30 - 12.70]	-0.33 (0.15) [-0.70 - -0.10]	-0.97, 0.31	0.154	(6.89 - 12.50) [5.51, 13.37]
Protein	32.70 (0.60) [31.48 - 34.13]	31.15 (0.60) [30.71 - 31.81]	1.55 (0.52) [0.55 - 2.32]	-0.70, 3.81	0.097	(29.51 - 40.25) [26.12, 43.51]
Total Fat	18.70 (0.50) [17.41 - 19.50]	19.88 (0.50) [19.38 - 20.32]	-1.18 (0.40) [-1.98 - -0.73]	-2.91, 0.56	0.099	(16.91 - 23.48) [15.35, 25.95]
Vitamin (mg/100g DW)						
Vitamin E	2.20 (0.22) [2.07 - 2.42]	2.91 (0.22) [2.58 - 3.49]	-0.72 (0.31) [-1.40 - -0.16]	-2.06, 0.63	0.148	(1.09 - 5.10) [0, 7.36]
Antinutrient						
Lectin (H.U./mg DW)	2.28 (0.63) [0.82 - 3.38]	2.46 (0.63) [1.92 - 3.41]	-0.18 (0.90) [-2.59 - 1.31]	-4.04, 3.67	0.855	(0.65 - 8.10) [0, 6.44]

Table E-10 (continued). Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Antinutrient (% DW)						
Phytic Acid	1.90 (0.072) [1.83 - 2.03]	1.88 (0.072) [1.72 - 1.97]	0.023 (0.076) [-0.13 - 0.11]	-0.30, 0.35	0.786	(1.42 - 2.27) [1.35, 2.35]
Raffinose	0.68 (0.021) [0.62 - 0.71]	0.68 (0.021) [0.66 - 0.70]	-0.0041 (0.029) [-0.064 - 0.051]	-0.13, 0.12	0.901	(0.40 - 0.80) [0.27, 0.87]
Stachyose	4.06 (0.24) [3.88 - 4.41]	4.10 (0.24) [3.50 - 4.43]	-0.038 (0.35) [-0.56 - 0.91]	-1.53, 1.45	0.922	(2.30 - 4.53) [1.96, 4.41]
Trypsin Inhibitor (TIU/mg DW)	40.79 (3.57) [33.98 - 47.66]	36.63 (3.57) [33.22 - 42.92]	4.16 (1.82) [0.76 - 6.98]	-3.67, 11.98	0.149	(23.11 - 60.42) [8.75, 63.43]
Isoflavone (µg/g DW)						
Daidzein	2481.08 (83.81) [2354.95 - 2565.56]	2406.95 (83.81) [2223.49 - 2565.86]	74.13 (58.64) [-43.14 - 134.06]	-178.17, 326.42	0.333	(320.54 - 3061.22) [0, 3328.03]
Genistein	1487.13 (52.05) [1433.45 - 1527.94]	1390.09 (52.05) [1254.28 - 1466.21]	97.04 (43.02) [33.79 - 179.17]	-88.04, 282.12	0.152	(433.41 - 2301.59) [0, 2727.33]

Table E-10 (continued). Statistical Summary of Site SFR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Isoflavone (µg/g DW)						
Glycitein	174.86 (13.28) [152.79 - 196.59]	122.44 (13.28) [98.05 - 146.12]	52.42 (18.78) [6.67 - 98.54]	-28.36, 133.21	0.107	(21.67 - 354.30) [0, 376.03]

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

Table E-11. Statistical Summary of Combined Site Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	30.18 (2.20) [20.04 - 42.14]	29.17 (2.23) [19.21 - 40.67]	1.01 (1.64) [-10.04 - 12.39]	-2.38, 4.39	0.543	(23.18 - 42.11) [18.29, 41.02]
Ash	8.75 (0.22) [7.39 - 10.11]	8.18 (0.22) [7.21 - 9.32]	0.57 (0.23) [-0.68 - 1.79]	0.096, 1.05	0.020	(6.76 - 10.40) [6.78, 9.91]
Carbohydrates	72.30 (1.20) [68.94 - 78.93]	73.43 (1.21) [67.88 - 78.99]	-1.13 (0.59) [-4.74 - 5.27]	-2.36, 0.097	0.069	(63.74 - 80.60) [64.45, 80.50]
Moisture (% FW)	72.93 (1.16) [70.20 - 81.10]	72.22 (1.16) [69.40 - 77.50]	0.70 (0.48) [-1.50 - 7.20]	-0.64, 2.04	0.218	(65.80 - 82.00) [62.26, 83.45]
Neutral Detergent Fiber	35.79 (2.05) [26.12 - 46.30]	35.85 (2.06) [32.19 - 45.90]	-0.062 (2.14) [-7.01 - 8.75]	-6.00, 5.88	0.978	(24.70 - 46.55) [22.57, 46.52]
Protein	14.04 (1.13) [9.25 - 18.10]	13.34 (1.13) [9.71 - 17.85]	0.70 (0.55) [-5.20 - 3.75]	-0.43, 1.84	0.213	(9.51 - 19.93) [7.38, 21.27]

Table E-11 (continued). Statistical Summary of Combined Site Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Total Fat	4.91 (0.42) [2.29 - 7.01]	5.14 (0.42) [3.36 - 7.19]	-0.23 (0.38) [-2.23 - 2.80]	-1.01, 0.55	0.549	(1.19 - 8.22) [0, 9.74]

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

Table E-12. Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Alanine	1.59 (0.033) [1.49 - 1.70]	1.57 (0.033) [1.43 - 1.66]	0.025 (0.018) [-0.041 - 0.13]	-0.026, 0.075	0.243	(1.34 - 1.78) [1.25, 1.92]
Arginine	2.78 (0.11) [2.43 - 3.16]	2.68 (0.11) [2.31 - 2.99]	0.10 (0.036) [-0.062 - 0.39]	0.0012, 0.20	0.048	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	4.08 (0.13) [3.67 - 4.49]	4.00 (0.13) [3.51 - 4.43]	0.075 (0.060) [-0.16 - 0.52]	-0.092, 0.24	0.279	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.61 (0.0075) [0.57 - 0.64]	0.59 (0.0076) [0.55 - 0.63]	0.022 (0.0075) [-0.027 - 0.073]	0.0010, 0.042	0.043	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	6.46 (0.24) [5.72 - 7.19]	6.32 (0.24) [5.42 - 7.09]	0.14 (0.12) [-0.32 - 1.02]	-0.19, 0.47	0.300	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.59 (0.039) [1.47 - 1.74]	1.56 (0.039) [1.41 - 1.67]	0.028 (0.022) [-0.050 - 0.17]	-0.033, 0.089	0.265	(1.30 - 1.79) [1.19, 1.95]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Alanine	1.59 (0.033) [1.49 - 1.70]	1.57 (0.033) [1.43 - 1.66]	0.025 (0.018) [-0.041 - 0.13]	-0.026, 0.075	0.243	(1.34 - 1.78) [1.25, 1.92]
Arginine	2.78 (0.11) [2.43 - 3.16]	2.68 (0.11) [2.31 - 2.99]	0.10 (0.036) [-0.062 - 0.39]	0.0012, 0.20	0.048	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	4.08 (0.13) [3.67 - 4.49]	4.00 (0.13) [3.51 - 4.43]	0.075 (0.060) [-0.16 - 0.52]	-0.092, 0.24	0.279	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.61 (0.0075) [0.57 - 0.64]	0.59 (0.0076) [0.55 - 0.63]	0.022 (0.0075) [-0.027 - 0.073]	0.0010, 0.042	0.043	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	6.46 (0.24) [5.72 - 7.19]	6.32 (0.24) [5.42 - 7.09]	0.14 (0.12) [-0.32 - 1.02]	-0.19, 0.47	0.300	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.59 (0.039) [1.47 - 1.74]	1.56 (0.039) [1.41 - 1.67]	0.028 (0.022) [-0.050 - 0.17]	-0.033, 0.089	0.265	(1.30 - 1.79) [1.19, 1.95]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Histidine	0.96 (0.023) [0.88 - 1.04]	0.94 (0.023) [0.84 - 1.01]	0.027 (0.010) [-0.021 - 0.11]	-0.0029, 0.057	0.065	(0.79 - 1.07) [0.74, 1.16]
Isoleucine	1.71 (0.050) [1.56 - 1.88]	1.67 (0.051) [1.45 - 1.86]	0.039 (0.036) [-0.15 - 0.26]	-0.064, 0.14	0.344	(1.37 - 2.00) [1.23, 2.15]
Leucine	2.73 (0.083) [2.47 - 3.01]	2.69 (0.083) [2.37 - 2.94]	0.044 (0.040) [-0.13 - 0.32]	-0.065, 0.15	0.325	(2.26 - 3.14) [2.06, 3.41]
Lysine	2.39 (0.053) [2.19 - 2.55]	2.33 (0.053) [2.10 - 2.51]	0.057 (0.025) [-0.051 - 0.25]	-0.011, 0.13	0.080	(2.00 - 2.63) [1.87, 2.81]
Methionine	0.55 (0.0088) [0.51 - 0.58]	0.53 (0.0089) [0.49 - 0.57]	0.020 (0.011) [-0.035 - 0.056]	-0.010, 0.049	0.141	(0.46 - 0.59) [0.43, 0.63]
Phenylalanine	1.82 (0.056) [1.64 - 2.00]	1.80 (0.056) [1.58 - 1.99]	0.019 (0.027) [-0.16 - 0.22]	-0.056, 0.093	0.523	(1.50 - 2.11) [1.35, 2.31]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Amino Acid (% DW)						
Proline	1.77 (0.057) [1.59 - 1.95]	1.72 (0.057) [1.52 - 1.90]	0.054 (0.030) [-0.046 - 0.27]	-0.029, 0.14	0.145	(1.43 - 2.03) [1.29, 2.21]
Serine	1.75 (0.059) [1.49 - 1.98]	1.77 (0.059) [1.45 - 1.94]	-0.016 (0.035) [-0.22 - 0.24]	-0.087, 0.056	0.655	(1.55 - 2.05) [1.44, 2.15]
Threonine	1.33 (0.030) [1.20 - 1.45]	1.33 (0.030) [1.18 - 1.47]	-0.0031 (0.018) [-0.13 - 0.11]	-0.041, 0.035	0.867	(1.19 - 1.48) [1.12, 1.53]
Tryptophan	0.42 (0.011) [0.37 - 0.46]	0.41 (0.012) [0.35 - 0.44]	0.0016 (0.0069) [-0.028 - 0.046]	-0.017, 0.020	0.831	(0.33 - 0.48) [0.30, 0.50]
Tyrosine	1.25 (0.029) [1.17 - 1.33]	1.22 (0.029) [1.10 - 1.32]	0.026 (0.014) [-0.040 - 0.12]	-0.011, 0.064	0.124	(1.07 - 1.39) [0.99, 1.49]
Valine	1.83 (0.052) [1.69 - 2.02]	1.77 (0.053) [1.55 - 1.96]	0.051 (0.038) [-0.17 - 0.28]	-0.059, 0.16	0.260	(1.45 - 2.13) [1.31, 2.29]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
16:0 Palmitic	2.36 (0.056) [2.25 - 2.44]	10.83 (0.056) [10.51 - 11.08]	-8.47 (0.055) [-8.68 - -8.13]	-8.62, -8.31	<0.001	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic	3.31 (0.067) [3.07 - 3.82]	4.50 (0.067) [4.24 - 4.85]	-1.19 (0.065) [-1.47 - -0.79]	-1.37, -1.01	<0.001	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic	76.47 (0.59) [73.13 - 79.17]	22.81 (0.59) [21.41 - 25.08]	53.65 (0.22) [51.71 - 55.05]	53.17, 54.13	<0.001	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic	10.10 (0.39) [7.85 - 12.42]	52.86 (0.39) [51.68 - 53.89]	-42.77 (0.18) [-43.74 - -41.38]	-43.17, -42.37	<0.001	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic	6.69 (0.28) [5.55 - 7.81]	8.02 (0.28) [6.86 - 8.60]	-1.33 (0.072) [-1.80 - -0.59]	-1.53, -1.13	<0.001	(5.89 - 9.11) [4.97, 9.93]
20:0 Arachidic	0.30 (0.0076) [0.28 - 0.36]	0.34 (0.0077) [0.31 - 0.36]	-0.039 (0.0071) [-0.082 - -0.022]	-0.059, -0.019	0.005	(0.28 - 0.54) [0.22, 0.53]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic	0.34 (0.013) [0.27 - 0.40]	0.19 (0.013) [0.15 - 0.21]	0.15 (0.0086) [0.12 - 0.20]	0.13, 0.18	<0.001	(0.15 - 0.22) [0.13, 0.25]
22:0 Behenic	0.29 (0.0037) [0.28 - 0.33]	0.30 (0.0038) [0.28 - 0.31]	-0.0052 (0.0051) [-0.029 - 0.020]	-0.017, 0.0070	0.346	(0.29 - 0.46) [0.22, 0.47]
24:0 Lignoceric	0.14 (0.017) [0.066 - 0.17]	0.13 (0.017) [0.067 - 0.16]	0.0046 (0.0046) [-0.019 - 0.027]	-0.0084, 0.018	0.372	(0.056 - 0.21) [0.030, 0.26]
Fiber (% DW)						
Acid Detergent Fiber	17.14 (0.54) [15.71 - 19.31]	16.14 (0.54) [13.36 - 18.02]	1.00 (0.74) [-1.84 - 4.03]	-1.06, 3.05	0.249	(12.46 - 21.25) [12.71, 19.29]
Neutral Detergent Fiber	18.44 (0.85) [13.41 - 22.18]	17.83 (0.86) [14.61 - 21.09]	0.60 (1.03) [-4.24 - 4.33]	-2.25, 3.46	0.590	(12.25 - 20.89) [12.07, 21.51]
Proximate (% DW)						
Ash	6.06 (0.13) [5.46 - 6.54]	6.13 (0.13) [5.48 - 6.55]	-0.072 (0.081) [-0.56 - 0.26]	-0.30, 0.15	0.421	(5.64 - 6.82) [5.26, 7.17]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Proximate (% DW)						
Carbohydrates	40.35 (0.86) [36.69 - 43.52]	39.93 (0.86) [37.46 - 42.96]	0.42 (0.37) [-1.08 - 2.00]	-0.59, 1.43	0.317	(32.79 - 42.29) [30.78, 45.86]
Moisture (% FW)	10.76 (0.37) [8.96 - 12.30]	11.56 (0.37) [10.20 - 12.70]	-0.80 (0.44) [-3.40 - 0.90]	-2.02, 0.42	0.141	(6.89 - 12.50) [5.51, 13.37]
Protein	35.32 (0.99) [31.48 - 38.59]	34.66 (0.99) [30.71 - 37.40]	0.66 (0.36) [-0.94 - 3.08]	-0.33, 1.65	0.141	(29.51 - 40.25) [26.12, 43.51]
Total Fat	18.29 (0.39) [16.55 - 19.50]	19.33 (0.39) [17.63 - 20.32]	-1.04 (0.16) [-2.16 - -0.20]	-1.39, -0.69	<0.001	(16.91 - 23.48) [15.35, 25.95]
Vitamin (mg/100g DW)						
Vitamin E	2.83 (0.43) [1.23 - 4.36]	3.27 (0.43) [1.69 - 4.19]	-0.44 (0.17) [-1.40 - 0.76]	-0.92, 0.037	0.062	(1.09 - 5.10) [0, 7.36]
Antinutrient						
Lectin (H.U./mg DW)	2.21 (0.40) [0.72 - 3.77]	2.45 (0.41) [0.61 - 5.53]	-0.24 (0.57) [-4.80 - 3.04]	-1.57, 1.09	0.686	(0.65 - 8.10) [0, 6.44]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Antinutrient (% DW)						
Phytic Acid	1.82 (0.052) [1.52 - 2.13]	1.85 (0.053) [1.63 - 2.19]	-0.031 (0.041) [-0.27 - 0.18]	-0.12, 0.057	0.457	(1.42 - 2.27) [1.35, 2.35]
Raffinose	0.58 (0.029) [0.48 - 0.71]	0.58 (0.029) [0.50 - 0.70]	0.00036 (0.015) [-0.088 - 0.12]	-0.042, 0.043	0.981	(0.40 - 0.80) [0.27, 0.87]
Stachyose	3.87 (0.13) [3.39 - 4.48]	3.70 (0.13) [3.04 - 4.43]	0.17 (0.14) [-0.56 - 0.96]	-0.22, 0.56	0.290	(2.30 - 4.53) [1.96, 4.41]
Trypsin Inhibitor (TIU/mg DW)	38.14 (2.60) [26.73 - 52.01]	37.25 (2.61) [27.23 - 49.78]	0.89 (0.97) [-5.31 - 6.98]	-1.74, 3.51	0.408	(23.11 - 60.42) [8.75, 63.43]
Isoflavone (µg/g DW)						
Daidzein	1806.33 (229.35) [1145.72 - 2565.56]	1794.07 (229.50) [1092.43 - 2565.86]	12.26 (37.50) [-196.53 - 242.28]	-68.88, 93.40	0.748	(320.54 - 3061.22) [0, 3328.03]
Genistein	1160.30 (115.82) [809.79 - 1527.94]	1117.27 (115.95) [751.67 - 1466.21]	43.04 (24.92) [-104.61 - 208.66]	-10.74, 96.81	0.107	(433.41 - 2301.59) [0, 2727.33]

Table E-12 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Isoflavone (µg/g DW)						
Glycitein	132.85 (12.38) [49.11 - 196.59]	126.86 (12.53) [72.10 - 167.04]	5.98 (12.18) [-61.67 - 98.54]	-27.41, 39.38	0.648	(21.67 - 354.30) [0, 376.03]

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

Table E-13. Literature and ILSI-CCD Ranges for Components in Soybean Forage and Seed

Forage Tissue/Component[1]	Literature Range[2]	ILSI-CCD 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	1.302 – 5.132
Fiber (% dw)		
Acid Detergent Fiber (ADF)	23.86 – 50.69	not available
Neutral Detergent Fiber (NDF)	19.61 – 43.70	not available

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 – 1.71	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

Table E-13 (continued). Literature and ILSI-CCD Ranges for Components in Soybean Forage and Seed

Seed Tissue Component[1]	Literature Range[2]	ILSI-CCD Range[3]
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
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
24:0 Lignoceric	0.13 – 0.24[4]	not available
Vitamins (mg/100g dw)		
Vitamin E	1.29 – 4.80	0.19-6.17
Antinutrients		
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
Bio-Actives (% dw)		
Raffinose	0.26 – 0.84	0.21 – 0.66
Stachyose	1.53 – 2.98	1.21 – 3.50

[1]fw=fresh weight; dw=dry weight; H.U. = hemagglutinating unit; TIU = trypsin inhibitor unit.
[2]Lundry et al. (2008). [3]ILSI Crop Composition Database at: http://www.cropcomposition.org. [4]Padgette et al. (1996). 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

E.6. Compositional Comparison of Processed Fractions from Soybean Seed of MON 87705 and the Conventional Control

To prepare soybean processed fractions, seed samples were collected from field trials conducted with MON 87705 and the conventional control at two field sites (Jefferson County, IA and Clinton County, IL) in the U.S. during the 2007 growing season. In addition, 12 commercial conventional soybean reference varieties were grown separately at three field sites in the U.S. to determine a 99% tolerance interval for each component analyzed. The seed samples were processed into defatted toasted soybean meal (TD soybean meal); refined, bleached, and deodorized soybean oil (RBD oil); protein isolate; and crude lecithin fractions. The processed fractions were analyzed according to the principles outlined in the OECD consensus document for soybean composition (OECD, 2001). Samples from all three replicates of MON 87705, the control, and the 12 references were analyzed from all plots. The TD soybean meal was analyzed for proximates (moisture, protein, fat, ash and carbohydrates by calculation), ADF, NDF, amino acids, trypsin inhibitors and phytic acid. The RBD oil was analyzed for fatty acids and vitamin E (α-tocopherol). The protein isolate fraction was analyzed for amino acids and moisture. The crude lecithin fraction was analyzed for phosphatides (α-phosphatidic acid, α phosphatidylcholine, α phosphatidylethanolamine, and α-phosphatidylinositiol).

Compositional analyses were conducted to assess whether the processed fractions prepared from MON 87705 are comparable to those of the conventional control, A3525, which has background genetics similar to MON 87705, but lacks the intentionally modified fatty acid profile and glyphosate tolerance traits. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods. In addition, 12 commercial conventional soybean varieties were grown and analyzed separately 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. The methods used for composition analysis of the processed fractions are summarized in Section E.7. The statistical analysis compared MON 87705 and the conventional control across the two sites (combined-site). A summary of the significant differences observed between the processed fractions prepared from the seed of MON 87705 and the conventional control is included in Table E-15. A statistical summary of the composition of each processed fraction is included in Tables E-16 to E-19. Literature ranges are provided in Table E-20 for soybean meal, Table E-21 for protein isolate, Table E-22 for soybean lecithin, and Table E-23 for soybean oil.

Results show that except for the intended changes in fatty acid composition, minor differences in the levels of less abundant fatty acids in RBD oil and occurrence of low levels of minor fatty acids due to spontaneous isomerisation during the oil refining process, the processed fractions produced from MON 87705 are compositionally equivalent to those of conventional soybean. Further details are provided below.

E.6.1. Composition of TD Soybean Meal

Comparison of the composition of TD soybean meal prepared from MON 87705 and the conventional soybean control showed no differences ($p>0.05$) for 21 of the 27 components analyzed. Significant differences ($p<0.05$) were observed for six

components (Table E-15): alanine, glycine, isoleucine, lysine, valine, and NDF. For the statistically significant amino acid differences, the absolute magnitude of the mean differences from the control were small (<0.1% dw) and the MON 87705 mean values fell within the 99% tolerance interval for the conventional soybean varieties and also within the range of published values for conventional soybean. For the statistically significant difference observed for NDF, the absolute magnitude of the mean difference from the control was small (1.7% dw) and the MON 87705 mean value was within the 99% tolerance interval for the conventional soybean varieties and also within the range of published values for conventional soybean (Table E-20). Therefore, these differences were not considered biologically relevant from a food and feed safety or nutritional perspective.

As expected, the TD soybean meal from MON 87705 contained a small amount of total fat or oil (0.78% dw), which was also present at similar levels (0.86% dw) in the meal from the conventional control (Table E-16). TD soybean meal is expected to contain oil, and accordingly, the National Oil Processors Association has established a minimum oil content (0.5% dw) for defatted soybean meal that meets quality standards and guidelines for soybean meal for domestic and international shipping (NOPA, 2006). Although not analyzed for, the composition of the residual oil present in TD soybean meal is expected be consistent with the intended fatty acid changes observed in seed and in RBD oil (see discussion in Section E.6.2 below). Based on these results, apart from the intended fatty acid changes in residual oil, the meal from MON 87705 soybean is considered compositionally equivalent to the meal from conventional soybean.

E.6.2. Composition of RBD Oil

Of the 38 fatty acids analyzed, 21 were excluded from statistical analysis since more than 50% of the observations were below the assay limit of quantitation. Of the 17 fatty acids that could be statistically analyzed, significant differences ($p<0.05$) between MON 87705 and control RBD oil were observed for 13 fatty acids (Table E-15). Four of the 13 differences were expected as they were due to the intended changes in fatty acid levels. Thus, 16:0 palmitic acid levels were significantly lower in MON 87705 (2.49% total FA) than the control (11.59% total FA), 18:0 stearic acid levels were significantly lower in MON 87705 (3.22% total FA) than the control (4.47% total FA); 18:1 oleic acid levels were significantly higher in MON 87705 (71.51% total FA) than the control (23.16% total FA), and 18:2 linoleic acid levels were significantly lower in MON 87705 (14.41% total FA) than the control (51.08% total FA) (Table E-15).

In addition to the intended changes, six fatty acids were detected in RBD oil that were not detected in seed: 14:0 myristic acid, 16:1 palmitoleic acid, 17:0 margaric (heptadecanoic) acid, 17:1 9c heptadecenoic acid, 18:2 other *trans* isomer fatty acids (excluding 9t,12t linolelaidic), and 18:2 6c,9c, octadecadienoic acid. As observed in seed, levels of several less abundant fatty acids were significantly different ($p<0.05$) between the RBD oil from MON 87705 and the conventional control. For six of the nine remaining fatty acids where a difference was observed (14:0 myristic acid, 16:1 palmitoleic acid, 17:0 margaric [heptadecanoic] acid, 20:0 arachidic, 20:1 eicosenoic and 22:0 behenic acids), the absolute magnitude of the differences was small (<0.15% total FA), and the MON 87705

mean values fell within the 99% tolerance intervals for the reference soybean varieties and/or within published ranges for conventional soybean oil (Codex, 2005).

A significant increase in the level of the minor fatty acid 17:1 9c heptadecenoic acid was observed in MON 87705 RBD oil (0.12% total FA) compared to its level in control RBD oil (0.031% total FA). This is not unexpected given the intended shift in fatty acid levels in MON 87705. The mean level of 17:1 9c heptadecenoic acid in MON 87705 RBD oil was higher than the level in the commercial reference varieties (<0.06% total FA) and slightly outside reported literature values for soybean oil (ND-0.1% total FA; Codex, 2005). However, 17:1 9c heptadecenoic acid is known to be present in other vegetables oils such as canola (0.3% total FA), corn (0.1% total FA), peanut (0.1% total FA), high oleic safflower (0.1% total FA), and high oleic sunflower (0.1% total FA) (Codex, 2005). The presence of 17:1 9c heptadecenoic acid has also been documented in a variety of foods, with levels highest in meats and oils. As shown in the Table E-14, comparable or higher intakes of 17:1 9c heptadecenoic acid can be achieved in a single serving of tofu, ground beef or soft-spread margarine compared to a serving of MON 87705 RBD oil. Therefore, it is concluded that there are no adverse food and feed safety or nutrition effects associated with the levels of 17:1 9c heptadecenoic acid observed in MON 87705 RBD oil.

Table E-14. Estimation of 17:1 9c Heptadecenoic Acid Intake in Selected Foods and MON 87705

Food	Level of 17:1 (g/100 g food)	RACC[1] (g)	Amount of 17:1 consumed per serving (mg/RACC)
Extra firm nigari tofu (16159)[2]	1.085	85	922.0
Ground beef, 80% lean (23572)[2]	0.135	85	115.0
Soft-spread margarine, 80% fat, canola based (04684)[2]	0.053	14	7.0
MON 87705	0.12	14	17.0

[1]Reference Amount Customarily Consumed, which is the U.S. serving size for labeling purposes (21CFR 101.12)

[2]Number in parentheses refers to Nutrient Database Number used to identify a particular food within the USDA-ARS food composition database.

The 18:2 other *trans* fatty acids and 18:2 6c,9c, octadecadienoic acid observed in both MON 87705 and the conventional control RBD oil were not observed in seed fatty acid analyses and are believed to arise from the spontaneous isomerization of unsaturated fatty acids during the oil refining process, particularly during the heat-requiring deodorization step (Chardigny et al. 1996). The primary source of industrially produced *trans* fatty acids (TFAs) in the human diet is the consumption of hydrogenated vegetable oils in liquid or solid form resulting in food products that may contain in excess of 30% TFA (Chardigny et al. 1996; Ledoux et al*,* 2007). Naturally occurring *trans* fats also arise as a result of bacterial reduction of unsaturated fatty acids in the gut of ruminant animals, with intake resulting from consumption of meat and dairy products (Chardigny et al. 1996; Ledoux et al*.,* 2007).

The mean level of 18:2 other *trans* fatty acids in MON 87705 RBD oil was significantly lower than in control RBD oil, and both values were outside the range of the commercial reference varieties. This is likely due to minor differences in processing methods as the commercial reference soybean varieties were grown and processed separately from MON 87705 and the conventional control. However, the levels of 18:2 other *trans* fatty acids in MON 87705 RBD oil were within the range of total TFA content in samples of unhydrogenated commercial soybean oil (<3.5%) (Chardigny et al*.,* 1996; Ledoux et al*.,* 2007; Wolff, 1993). The contribution of MON 87705 RBD oil to overall dietary TFA intake will be minimal relative to commonly experienced dietary intakes, and therefore, are not biologically relevant from a food and feed safety or nutritional perspective.

The mean level of 18:2 6c,9c, octadecadienoic acid in MON 87705 RBD oil was significantly lower than in conventional control RBD oil, and both values were outside the range of the commercial references. This is likely due to minor differences in processing methods as the commercial reference soybean varieties were grown and processed separately from MON 87705 and the conventional control. It has been reported that although most fatty acid double bonds are in the *cis* configuration, some processes (such as heat treatment) may lead to the migration of double bonds from their naturally occurring positions in the carbon chain, leading to an increase in the levels of other cis isomers (Ledoux et al., 2007).

The RBD oil was also analyzed for vitamin E levels which were not significantly different ($p>0.05$) between MON 87705 and conventional control RBD oil.

In summary, except for intended changes in fatty acid composition, minor differences in the levels of less abundant fatty acids and occurrence of low levels of minor fatty acids due to spontaneous isomerisation during the oil refining process, the RBD oil from MON 87705 is considered compositionally equivalent to oil from conventional soybean.

E.6.3. Composition of Soybean Protein Isolate

There were no statistically significant differences ($p<0.05$) between MON 87705 and the conventional control for components measured in the protein isolate fraction. Statistical summary data on the composition of the soybean protein isolate fraction is included in Table E-18. Based on these results, the protein isolate prepared from MON 87705 is considered compositionally equivalent to protein isolate from conventional soybean.

E.6.4. Composition of Crude Lecithin

There were no statistically significant differences ($p<0.05$) between MON 87705 and the conventional control for phosphatides of crude lecithin. Statistical summary data on the composition of the crude lecithin fraction is included in Table E-19. Based on these results, the MON 87705 soybean lecithin is considered compositionally equivalent to conventional soybean lecithin.

E.6.5. Compositional Equivalence of MON 87705 and Conventional Soybean Processed Fractions

The processed fractions, TD soybean meal, RBD oil, protein isolate and crude lecithin, were analyzed according to the principles outlined in the OECD consensus document for soybean composition (OECD, 2001). There were no statistically significant differences ($p<0.05$) observed between MON 87705 and the conventional control (A3525) for the components measured in protein isolates or crude lecithin. Significant differences ($p<0.05$) were observed for six (alanine, glycine, isoleucine, lysine, valine, and NDF) of 27 components measured in TD soybean meal; however, the magnitude of the differences from the conventional control was small and the MON 87705 mean values were within the 99% tolerance interval for the conventional reference soybean varieties and/or within the range of published values for conventional soybean meal, indicating that they were not biologically meaningful. The low levels of oil (0.78% dw, as total fat) present in TD soybean meal are expected to reflect the intended fatty acid changes observed in seed.

As expected, and consistent with results obtained for seed fatty acid levels, the intended fatty acid changes were observed in the RBD oil fraction from MON 87705. As observed in seed, levels of several less abundant fatty acids were significantly different ($p<0.05$) between the RBD oil from MON 87705 and the conventional control. Of the 17 fatty acids that could be statistically analyzed in RBD oil, significant differences ($p<0.05$) between MON 87705 and the conventional control were observed for 13 fatty acids. Four of the 13 differences were expected as they were due to the intended changes in fatty acid levels (16:0 palmitic, 18:0 palmitic, 18:1 oleic and 18:2 linoleic). For six of the nine remaining differences (14:0 myristic acid, 16:1 palmitoleic acid, 17:0 margaric [heptadecanoic] acid, 20:0 arachidic, 20:1 eicosenoic and 22:0 behenic acids), the magnitude of the differences was small (<0.15% total FA), and the MON 87705 mean values fell within the 99% tolerance intervals for the reference varieties and/or within published ranges for conventional RBD soybean oil (Codex, 2005, Table E-23).

The remaining three differences were for 17:1 9c heptadecenoic acid, 18:2 *trans* isomer fatty acids (excluding linolelaidic), and 18:2 6c,9c, octadecadienoic acid. A significant increase in the level of the minor fatty acid 17:1 9c heptadecenoic acid was observed in MON 87705 compared to conventional control RBD oil. This is not unexpected given the intended shift in fatty acid levels in MON 87705. The mean level of 17:1 9c heptadecenoic acid in MON 87705 was outside the range of values obtained for the RBD oil from commercial reference soybean varieties. However, 17:1 9c heptadecenoic acid is present at similar or higher levels in a variety of oils (canola, corn, peanut, high oleic safflower, and high oleic sunflower) and foods (tofu, ground beef, and soft-spread margarine). Therefore, it is concluded that there are no adverse food and feed safety or nutrition effects associated with the levels of 17:1 9c heptadecenoic acid observed in MON 87705 soybean oil. The minor fatty acids, 18:2 other *trans* (excluding linolelaidic) and 18:2 6c,9c, octadecadienoic acid, are believed to arise from the spontaneous isomerization of unsaturated fatty acids during the oil refining process.

Therefore, this compositional assessment supports the conclusion that, except for the intended changes in fatty acid composition, minor differences in the levels of less abundant fatty acids and occurrence of low levels of minor fatty acids due to spontaneous isomerisation during the oil refining process, the processed fractions produced from MON 87705 are compositionally equivalent to those of conventional soybean.

Table E-15. Summary of Differences (p<0.05) for the Comparison of Soybean Processed Fraction Component Levels for MON 87705 vs. the Conventional Control (A3525) and Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	Mean Difference (MON 87705 minus Control) Signif. (p-Value)	Test Range	Commercial Range[2] (99% Tolerance Interval)[2,3]
Meal Amino Acid (% DW)						
Alanine	2.26	2.33	-3.06	0.019	2.22 - 2.29	2.18 – 2.49 (1.87, 2.74)
Glycine	2.28	2.34	-2.44	0.023	2.26 - 2.33	2.19 – 2.46 (1.91, 2.68)
Isoleucine	2.45	2.53	-3.17	0.006	2.40 - 2.51	2.36 – 2.71 (2.03, 3.06)
Lysine	3.25	3.34	-2.49	0.030	3.22 - 3.29	3.07 – 3.48 (2.65, 3.85)
Valine	2.55	2.64	-3.47	0.003	2.51 - 2.60	2.48 – 2.91 (2.07, 3.26)
Meal Fiber (% DW)						
Neutral Detergent Fiber	8.55	6.81	25.47	0.016	8.05 - 8.96	6.20 – 10.58 (2.19, 13.59)
RBD Oil Fatty Acid (% Total FA)						
14:0 Myristic	0.031	0.090	-65.15	<0.001	0.031 - 0.032	0.066 – 0.11 (0.024, 0.14)
16:0 Palmitic	2.49	11.59	-78.50	<0.001	2.36 - 2.69	9.22 – 11.96 (7.75, 13.82)
16:1 Palmitoleic	0.13	0.11	22.10	0.012	0.12 - 0.14	0.072 – 0.11 (0.044, 0.14)
17:0 Margaric [Heptadecanoic]	0.036	0.10	-65.27	0.002	0.031 - 0.048	0.047 – 0.10 (0.0082, 0.16)
17:1 9c Heptadecenoic	0.12	0.031	279.62	0.006	0.092 - 0.14	0 < 0.02 not calculated
18:0 Stearic	3.22	4.47	-28.05	<0.001	3.00 - 3.40	3.58 – 5.00 (1.83, 6.48)

Table E-15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Processed Fraction Component Levels for MON 87705 vs. the Conventional Control (A3525) and Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control)		Test Range	Commercial Range[2] (99% Tolerance Interval)[2]
			Mean Difference (% of A3525)	Signif. (p-Value)		
RBD Oil Fatty Acid (% Total FA)						
18:1 Oleic	71.51	23.16	208.71	<0.001	69.30 - 73.01	21.10 – 31.19 (11.72, 37.78)
18:2 6c,9c Octadecadienoic	0.20	0.65	-69.86	<0.001	0.16 - 0.24	0.031 – 0.074 (0, 0.13)
18:2 Linoleic	14.41	51.08	-71.78	<0.001	12.25 - 17.39	47.74 – 53.88 (42.34, 61.19)
18:2 Other Trans	0.18	0.63	-70.92	<0.001	0.14 - 0.23	0 < 0.02 Not calculated
20:0 Arachidic	0.29	0.36	-19.97	0.001	0.27 - 0.31	0.28 – 0.43 (0.13, 0.59)
20:1 11c Eicosenoic	0.33	0.19	73.17	<0.001	0.29 - 0.37	0.18 – 0.27 (0.066, 0.37)
22:0 Behenic	0.31	0.35	-9.74	0.001	0.30 - 0.35	0.30 – 0.50 (0.11, 0.70)

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

E.7. Methods and Materials for Compositional Analysis of Processed Fractions

MON 87705 and a conventional soybean control (A3525) were grown at two U.S. locations in 2007. MON 87705 was grown from seed lots GLP-0704-18516-S and GLP-0705-18715-S, and the conventional soybean control was grown from seed lots GLP-0704-18512-S and GLP-0705-18716-S. The conventional soybean control has background genetics representative of MON 87705 but does not contain *cp4 epsps* or the *FATB1-A* and *FAD2-1A* gene segments. In addition, 12 commercial conventional soybean varieties produced at three locations in separate U.S. field trials in 2007 were included for the generation of a 99% tolerance interval. The conventional varieties, locations, and seed lot numbers are listed below:

Material Name	Starting Seed Lot No.	Field Site
Anand	GLP-0705-18678-S	AR
UA4805	GLP-0705-18679-S	AR
Ozark	GLP-0705-18680-S	AR
Delta & Pine 5989	GLP-0705-18681-S	AR
H437	GLP-0705-18686-S	IL
NK S38-T8	GLP-0705-18687-S	IL
LG C3540	GLP-0705-18688-S	IL
HS38C60	GLP-0705-18689-S	IL
NuPride 2954	GLP-0705-18682-S	NE
NC+ 2A86	GLP-0705-18683-S	NE
Pioneer 92B72	GLP-0705-18684-S	NE
NK 25-J5	GLP-0705-18685-S	NE

E.7.1. Characterization of the Materials

The identities of MON 87705, the conventional soybean control, and conventional reference soybean varieties were verified prior to use by confirming the chain-of-custody documentation of the samples from the field cooperators. The seed samples from MON 87705 and the conventional soybean control were further characterized by an event-specific PCR analysis of the DNA extracted from the seed to confirm the presence or absence of MON 87705.

E.7.2. Field Production of the Samples

Seed of the MON 87705 and the conventional soybean control were collected from two plots at each of two sites across the U.S. during the 2007 growing season. The field sites were in Clinton County, IL and Jefferson County, IA. The reference varieties were produced separately from one plot grown at one of three sites in the U.S. in 2007. The reference soybean varieties were planted at field sites in Jackson County, AR, Clinton County, IL, and York County, NE. All the reference varieties were grown under normal agronomic field conditions for their respective geographic regions. Seed was shipped at ambient temperature from all production locations to Monsanto Company, St. Louis, MO, USA. A subsample for compositional analyses was obtained from each seed sample. The seed subsamples were ground and stored in a freezer set to maintain -20° C at Monsanto Company (St. Louis, MO) prior to transfer to Covance Laboratories, Inc. (Madison, WI). The labels on the samples shipped to Covance laboratories Inc. listed the Monsanto study number, crop and sample type, production site and plot number, storage conditions, unique sample ID, lot or source number, material name, container type, and contact name.

Additionally, a subsample of each MON 87705, conventional control and reference seed sample was shipped from Monsanto Company (St. Louis, MO) to GLP-Technologies (GLP-T) in Navasota, Texas, for processing into TD soybean meal, refined, RBD oil, protein isolate and crude lecithin. A subsample for use in compositional analysis was obtained from each processed sample generated at GLP-T, and was shipped on dry ice to Monsanto Company (St. Louis, MO). The sample containers were relabeled at Monsanto with the following information: Monsanto study number, processing site, sample identifier, lot number, crop and sample type, material name, container type, contact name, and storage conditions, and then shipped overnight on dry ice to Covance Laboratories Inc. (Madison, WI) for analyses.

E.7.3. Compositional Analysis Methods for Processed Fractions

The compositional analysis methods for ADF, amino acids, ash, carbohydrates, fat, isoflavones, lectin, moisture, NDF, phytic acid, protein, and vitamin E were the same as described in Section E.6. Methods for analytes that had different standards and/or detection limits and the method for phosphatides, are described below.

E.7.3.1. Fatty Acid Profile with Trans Fat by GC

The lipid was extracted, saponified with 0.5N methanolic sodium hydroxide, and methylated with 14% BF_3-methanol. The resulting methyl esters of the fatty acids were extracted with heptane. An internal standard was added prior to the lipid extraction. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The limit of quantitation was 0.0200% – 0.0600% depending on the matrix.

- Nu Chek Prep GLC Reference Standard Hazelton No. 1, Lot Number JY19-R, 100%*
- Nu Chek Prep GLC Reference Standard Hazelton No. 2, Lot Number M13-O, 100%*
- Nu Chek Prep GLC Reference Standard Hazelton No. 3, Lot Number MA18-S, 100%*
- Nu Chek Prep GLC Reference Standard Hazelton No. 4, Lot Number JY19-R, 100%*
- Nu Chek Prep Methyl Gamma Linolenate, used as 100% , Lot Number U-63M-JY12-R
- Nu Chek Prep Methyl Tridecanoate, used as 100%, Lot Number N-13M-F5-S
- Nu Chek Prep Methyl Butyrate, used as 100%, Lot Number N-4M-J20-R
- Nu Chek Prep Methyl Hexanoate, used as 100%, Lot Number N-6M-A25-R
- Nu Chek Prep Methyl Erucate, used as 100%, Lot Number U-79M-AU3-Q
- Nu Chek Prep Methyl Lignocerate, used as 100%, Lot Number N-24M-F5-S
- Nu Chek Prep Methyl Docosapentaenoate, used as 100%, Lot Number U-101M-F18-S
- Nu Chek Prep Methyl Docosahexaenoate, used as 100%, Lot Number U-84M-D24-R
- Nu Chek Prep Methyl Eicosapentaenoate, used as 100%, Lot Number U-99M-D14-R
- Cayman Chemicals Steariodonic Acid Methyl Ester, used as 100%, Lot Number 186208-192001 and 186208-192002
- Nu Chek Prep Methyl Elaidate, used as 100%, Lot Number U-47M-JA18-R
- Nu Chek Prep Methyl Linoelaidate, used as 100%, Lot Number U-60M-F27-R
- Nu Chek Prep Methyl Nervonate, used as 100%, Lot Number U-88M-O19-R
- Nu Chek Prep Methyl Palmitelaidate, used as as 100%, Lot Number U-41M-O26-R
- Monsanto Mono Trans SDA, 99%, Lot Number GLP-0804-19309-A
- Monsanto Alpha Linolenic Acid, used as 100%, Lot Number GLP-0804-19308-A
- Monsanto 9c, 15c Octadecadienoate (Omnisoy), used as 100%, Lot Number GLP-0802-19168-A
- Larodan Methyl 6(z), 9(z)-Octadecadienoate, used as 99.6%, Lot Number LS-113
- Monsanto Omnisoy C17:1 Methyl 9-cis-Heptadecenoate, used as 99%, Lot Number GLP-0806-19436-A

* Overall purity of the sum of the mixture components

Literature Reference

AOCS. 1997. Method Ce 1-62 in Official Methods and Recommended Practices of the AOCS, 5th ed. American Oil Chemists' Society, Champaign, IL.

E.7.3.2. Isoflavones Analysis

The sample was extracted using a solution of hydrochloric acid and reagent alcohol heated on steam baths or hot plates. The extract was brought to volume, diluted, and centrifuged. An aliquot of the supernatant was placed onto a C18 solid-phase extraction column. Unwanted components of the matrix were rinsed off with 20% methanol and then the isoflavones were eluted with 80% methanol. The sample was analyzed on a HPLC system with ultraviolet detection and was compared to an external standard curve of known standards for quantitation. The limit of quantitation for each component was 10.0 µg/g.

Reference Standards

- Indofine, Daidzein, 99%, Lot Number 020508146

Indofine, Genistein, ≥99%, (used as 100%), Lot Number 0309074

Indofine, Glycitein, 99%, Lot Number 0704034

Literature References

Seo, A. and C.V. Morr. 1984. Improved high-performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. Journal of Agricultural and Food Chemistry, 32(3):530-533.

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

E.7.3.3. Phosphatides

The sample was extracted with a 98% $CHCl_3$ 2% MeOH solvent. The extract was then analyzed on an HPLC system equipped with an evaporative light-scattering detector (ELSD). A calibration curve was used for quantification. The LOQs for these assays were as follows: L-alpha-Phosphatidic Acid 0.70%, L-alpha-Phosphatidylcholine 1.30%, L-alpha-Phosphatidylethanolamine 1.30%, and L-alpha-Phosphatidylinositol 0.70%.

Reference Standards

- (PA) – Avanti Polar Lipids, L-alpha-Phosphatidic Acid (sodium salt), 100%, Lot Numbers SPA-19 and SPA-20.
- (PC) – Avanti Polar Lipids, L-alpha-Phosphatidylcholine, 100%, Lot Numbers PPC-116f and PPC-117

- (PE) – Avanti Polar Lipids, L-alpha-Phosphatidylethanolamine, 100%, Lot Numbers PPE-133 and PPE-133c.
- (PI) – Avanti Polar Lipids, L-alpha-Phosphatidylinositol (Sodium salt), 100%, Lot Numbers PPI-151 and PPI-154.

Literature Reference

AOCS Official Method Ja 7b-91. 1997. Determination of Lecithin Phospholipids by HPLC.

E.7.3.4. Raffinose and Stachyose

The sample was extracted with deionized water and the extract treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl-β-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography using a flame ionization detector. The limit of quantitation was 0.0500%.

Reference standards

- Sigma, Raffinose Pentahydrate, 99% / 84.0% after correction for degree of hydration, Lot Number 035K1371
- Sigma, Stachyose, 98% / 96.4% after correction for degree of hydration, Lot Number 065K3775

Literature References

Brobst, K. M. 1972. Gas-Liquid Chromatography of Trimethylsilyl Derivatives in Methods in Carbohydrate Chemistry, Vol. 6. Academic Press, New York.

Mason, B.S., and H.T. Slover. 1971. A Gas Chromatographic Method for the Determination of Sugars in Foods. Journal of Agricultural and Food Chemistry, 19(3):551-554.

E.7.3.5. Trypsin Inhibitor

The sample was ground and defatted with petroleum ether. A sample of matrix was extracted for three hours with 0.01 N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and benzoy1-DL-arginine~p~nitroanilide hydrochloride. The sample was allowed to react for 10 minutes at 37°C. After 10 minutes, the reaction was halted by the addition of acetic acid. The solution was centrifuged, then the absorbance was determined at 410 nm. Trypsin inhibitor activity was determined by photometrically measuring the inhibition of trypsin's reaction with benzoyl-DL-arginine~p~nitroanilide hydrochloride. The limit of quantitation was 1.00 Trypsin Inhibitor Units (TIU)/mg.

Literature Reference

AOCS. 1997. Method Ba 12-75 in Official Methods and Recommended Practices of the American Oil Chemists' Society. AOCS Press, Champaign, IL.

E.8 Data Processing

After compositional analyses were performed at Covance Laboratories Inc., data spreadsheets were forwarded to Monsanto Company. The data were reviewed, formatted, and sent to Certus International, Inc. for statistical analysis. A statistical sub-report was generated by Certus International, Inc. and sent to Monsanto Company. In all, 27 components were analyzed in meal, 39 in RBD oil, 19 in protein isolates, and four in lecithin. The following RBD oil components were excluded from statistical analysis: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 10c pentadecenoic acid, 16:1 palmitelaidic, 18:1 elaidic acid, 18:2 9c,15c octadecadienoic acid, 18:2 linolelaidic acid, 18:3 gamma linolenic acid, 18:4 6c,9c,12c,15t trans-SDA, 18:4 stearidonic acid, 20:2 11c, 14c eicosadienoic acid, 20:3 11c,14,17c eicosatrienoic acid, 20:4 arachidonic acid, 20:5 5c,8c,11c,14c,17c eicosapentaenoic acid, 22:1 erucic acid, 22:5 7c,10c,13c,16c,19c docosapentaenoic acid, 22:6 4c,7c,10c,13c,16c,19c docosehexaenoic acid, and 24:1 nervonic acid. The LOQ for the oil fatty acid method was 0.0600% fresh weight.

The following observations were below the LOQ: five observations for trypsin inhibitor in meal; eight observations for 14:0 myristic acid, seven observations for 17:0 heptadecenoic acid, and eight observations for 17:1 9c heptadecenoic acid in RBD oil; four observations for L-alpha-phosphatidic acid and two observations for L-alpha-phosphatidylethanolamine in lecithin. These observations were assigned a value equal to half the LOQ for the respective assay.

The data were assessed for potential outliers using a studentized PRESS residuals calculation. No values in the data set were removed as outliers.

E.9. Statistical Methodology

The compositional components for MON 87705 and the conventional soybean control were statistically analyzed across all sites using a mixed-model analysis of variance. The combined-site analyses used the model:

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

where Y_{ij} = unique individual observation, U = overall mean, T_i = substance effect, B_j = random replication block effect, and e_{ij} = residual error. For the processed fractions compositional components, MON 87705 was compared to the conventional soybean control.

A range of observed values from the reference soybean varieties was determined for each analytical component. Additionally, the reference soybean varieties' data were used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional reference soybean varieties (Ridley et al., 2002; George et al., 2004). Each tolerance interval estimate was based upon one observation per unique reference variety. A single replicate from each unique reference variety was analyzed for inclusion in tolerance interval calculations. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero. SAS programming was used to generate all summary statistics and perform all analyses (SAS Institute 2002-2003). Report tables present p-values from SAS as either <0.001 or the actual value truncated to three decimals.

Table E-16. Comparison of Amino Acid, Fiber, Proximate, and Antinutrient Content from MON 87705 and Conventional Control (A3525) Soybean Meal Processed from a 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E.[1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial Range [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine	2.26 (0.021) [2.22 - 2.29]	2.33 (0.021) [2.26 - 2.38]	-0.071 (0.016)	-0.12, -0.022	0.019	2.18 - 2.49 [1.87, 2.74]
Arginine	4.22 (0.074) [4.01 - 4.37]	4.31 (0.074) [4.17 - 4.49]	-0.091 (0.075)	-0.33, 0.15	0.312	3.83 - 4.84 [2.79, 5.46]
Aspartic Acid	5.95 (0.068) [5.83 - 6.14]	6.16 (0.068) [5.99 - 6.32]	-0.21 (0.066)	-0.42, 0.0058	0.053	5.69 - 6.63 [4.80, 7.35]
Cystine	0.82 (0.028) [0.75 - 0.90]	0.81 (0.028) [0.78 - 0.87]	0.0016 (0.019)	-0.059, 0.062	0.938	0.69 - 0.82 [0.56, 0.94]
Glutamic Acid	9.43 (0.11) [9.17 - 9.70]	9.78 (0.11) [9.48 - 9.98]	-0.35 (0.14)	-0.79, 0.091	0.086	9.05 - 10.63 [7.62, 11.74]
Glycine	2.28 (0.018) [2.26 - 2.33]	2.34 (0.018) [2.29 - 2.38]	-0.057 (0.013)	-0.099, -0.015	0.023	2.19 - 2.46 [1.91, 2.68]
Histidine	1.40 (0.019) [1.38 - 1.43]	1.44 (0.019) [1.39 - 1.50]	-0.041 (0.016)	-0.093, 0.012	0.089	1.29 - 1.62 [0.99, 1.88]
Isoleucine	2.45 (0.021) [2.40 - 2.51]	2.53 (0.021) [2.50 - 2.58]	-0.080 (0.012)	-0.12, -0.043	0.006	2.36 - 2.71 [2.03, 3.06]

Table E-16 (continued). Comparison of Amino Acid, Fiber, Proximate, and Antinutrient Content from MON 87705 and Conventional Control (A3525) Soybean Meal Processed from a 2007 U.S. Field Production

			Difference (MON 87705 minus Control)			
Analytical Component[1]	MON 87705 Mean ± S.E. [1] [Range]	A3525 Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine	4.02 (0.038) [3.94 - 4.13]	4.14 (0.038) [4.04 - 4.22]	-0.11 (0.038)	-0.23, 0.0060	0.056	3.92 - 4.39 [3.41, 4.84]
Lysine	3.25 (0.019) [3.22 - 3.29]	3.34 (0.019) [3.28 - 3.39]	-0.083 (0.022)	-0.15, -0.015	0.030	3.07 - 3.48 [2.65, 3.85]
Methionine	0.82 (0.024) [0.76 - 0.89]	0.84 (0.024) [0.79 - 0.89]	-0.021 (0.025)	-0.10, 0.057	0.452	0.71 - 0.80 [0.65, 0.86]
Phenylalanine	2.70 (0.030) [2.65 - 2.78]	2.79 (0.030) [2.71 - 2.86]	-0.093 (0.032)	-0.19, 0.0074	0.060	2.68 - 3.02 [2.28, 3.29]
Proline	2.65 (0.040) [2.60 - 2.76]	2.76 (0.040) [2.65 - 2.85]	-0.11 (0.037)	-0.23, 0.0099	0.061	2.48 - 2.87 [2.15, 3.08]
Serine	2.64 (0.037) [2.58 - 2.69]	2.73 (0.037) [2.61 - 2.81]	-0.093 (0.050)	-0.25, 0.065	0.157	2.50 - 2.84 [2.25, 3.02]
Threonine	2.06 (0.027) [1.99 - 2.09]	2.13 (0.027) [2.05 - 2.18]	-0.070 (0.031)	-0.17, 0.028	0.106	1.89 - 2.09 [1.73, 2.24]
Tryptophan	0.60 (0.013) [0.57 - 0.62]	0.60 (0.013) [0.57 - 0.64]	-0.0030 (0.013)	-0.045, 0.039	0.835	0.55 - 0.64 [0.47, 0.72]

Table E-16 (continued). Comparison of Amino Acid, Fiber, Proximate, and Antinutrient Content from MON 87705 and Conventional Control (A3525) Soybean Meal Processed from a 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E. [1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial Range [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine	1.83 (0.023) [1.78 - 1.85]	1.85 (0.023) [1.80 - 1.91]	-0.027 (0.021)	-0.093, 0.039	0.279	1.55 - 1.97 [1.29, 2.26]
Valine	2.55 (0.020) [2.51 - 2.60]	2.64 (0.020) [2.61 - 2.69]	-0.092 (0.010)	-0.13, -0.058	0.003	2.48 - 2.91 [2.07, 3.26]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	6.54 (0.26) [5.96 - 6.89]	5.94 (0.26) [5.34 - 6.70]	0.59 (0.37)	-0.30, 1.49	0.156	4.34 - 8.07 [1.27, 10.32]
Neutral Detergent Fiber	8.55 (0.26) [8.05 - 8.96]	6.81 (0.26) [6.26 - 7.50]	1.74 (0.36)	0.59, 2.88	0.016	6.20 - 10.58 [2.19, 13.59]
Ash	6.99 (0.17) [6.74 - 7.30]	7.29 (0.17) [6.88 - 7.76]	-0.29 (0.097)	-0.60, 0.014	0.056	6.36 - 7.45 [5.71, 8.17]
Carbohydrates	39.31 (0.71) [37.73 - 40.04]	37.69 (0.71) [35.68 - 39.61]	1.62 (0.59)	-0.25, 3.49	0.070	32.34 - 41.36 [27.18, 48.54]
Moisture (% FW)	3.33 (1.41) [2.72 - 3.91]	6.86 (1.41) [3.94 - 12.50]	-3.53 (2.00)	-8.41, 1.35	0.127	3.74 - 12.65 [0, 21.82]

Table E-16 (continued). Comparison of Amino Acid, Fiber, Proximate, and Antinutrient Content from MON 87705 and Conventional Control (A3525) Soybean Meal Processed from a 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E.[1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Protein	52.91 (0.59) [52.01 - 54.17]	54.16 (0.59) [52.52 - 55.85]	-1.24 (0.63)	-3.24, 0.76	0.142	51.40 - 59.42 [44.28, 64.08]
Total Fat	0.78 (0.084) [0.61 - 1.04]	0.86 (0.084) [0.69 - 1.00]	-0.079 (0.10)	-0.41, 0.25	0.495	0.65 - 1.72 [0, 2.12]
Antinutrient (% DW)						
Phytic Acid	1.37 (0.033) [1.27 - 1.43]	1.47 (0.033) [1.44 - 1.54]	-0.10 (0.033)	-0.21, 0.0047	0.055	1.06 - 1.53 [0.67, 1.90]
Trypsin Inhibitor (TIU/mg DW)	2.15 (0.59) [0.52 - 4.09]	1.41 (0.59) [0.54 - 2.07]	0.74 (0.83)	-1.29, 2.77	0.405	ND NC

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval; ND = not detected; NC = not calculated.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table E-17. Comparison of Fatty Acids and Vitamin E from MON 87705 and Conventional Control (A3525) Soybean RBD Oil Processed from the 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E. [1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial Range [99% Tolerance Int.[2]]
RBD Oil Fatty Acid (% Total FA)						
14:0 Myristic	0.031 (0.0017) [0.031 - 0.032]	0.090 (0.0017) [0.086 - 0.097]	-0.059 (0.0025)	-0.065, -0.053	<0.001	0.066 - 0.11 [0.024, 0.14]
16:0 Palmitic	2.49 (0.087) [2.36 - 2.69]	11.59 (0.087) [11.36 - 11.83]	-9.10 (0.087)	-9.37, -8.82	<0.001	9.22 - 11.96 [7.75, 13.82]
16:1 Palmitoleic	0.13 (0.0044) [0.12 - 0.14]	0.11 (0.0044) [0.096 - 0.11]	0.023 (0.0044)	0.0094, 0.037	0.012	0.072 - 0.11 [0.044, 0.14]
17:0 Margaric	0.036 (0.0070) [0.031 - 0.048]	0.10 (0.0070) [0.085 - 0.12]	-0.067 (0.0073)	-0.091, -0.044	0.002	0.047 - 0.10 [0.0082, 0.16]
17:1 9c Heptadecenoic	0.12 (0.0090) [0.092 - 0.14]	0.031 (0.0090) [0.031 - 0.031]	0.088 (0.013)	0.047, 0.13	0.006	ND NC
18:0 Stearic	3.22 (0.072) [3.00 - 3.40]	4.47 (0.072) [4.33 - 4.57]	-1.25 (0.060)	-1.44, -1.06	<0.001	3.58 - 5.00 [1.83, 6.48]
18:1 Oleic	71.51 (0.85) [69.30 - 73.01]	23.16 (0.85) [21.44 - 25.54]	48.35 (1.21)	45.39, 51.30	<0.001	21.10 - 31.19 [11.72, 37.78]
18:2 6c,9c Octadecadienoic	0.20 (0.017) [0.16 - 0.24]	0.65 (0.017) [0.63 - 0.69]	-0.46 (0.024)	-0.52, -0.40	<0.001	0.031 - 0.074 [0, 0.13]

Table E-17 (continued). Comparison of Fatty Acids and Vitamin E from MON 87705 and Conventional Control (A3525) Soybean RBD Oil Processed from the 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E. [1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
RBD Oil Fatty Acid (% Total FA)						
18:2 Linoleic	14.41 (0.92) [12.25 - 17.39]	51.08 (0.92) [50.02 - 52.06]	-36.67 (1.18)	-40.44, -32.90	<0.001	47.74 - 53.88 [42.34, 61.19]
18:2 Other Trans	0.18 (0.017) [0.14 - 0.23]	0.63 (0.017) [0.60 - 0.66]	-0.44 (0.024)	-0.50, -0.39	<0.001	ND NC
18:3 9c,12c,15t Octadecatrienoic	0.92 (0.073) [0.81 - 1.01]	0.98 (0.073) [0.80 - 1.19]	-0.059 (0.045)	-0.20, 0.085	0.284	0.031 - 0.13 [0, 0.22]
18:3 Linolenic	4.76 (0.38) [4.08 - 5.46]	5.13 (0.38) [4.21 - 5.96]	-0.37 (0.15)	-0.87, 0.12	0.094	5.07 - 9.34 [1.19, 12.72]
18:3 Other 18:3 Trans	0.92 (0.067) [0.81 - 1.01]	0.93 (0.067) [0.78 - 1.16]	-0.011 (0.045)	-0.15, 0.13	0.815	0.031 - 0.092 [0, 0.17]
20:0 Arachidic	0.29 (0.0088) [0.27 - 0.31]	0.36 (0.0088) [0.34 - 0.38]	-0.072 (0.0060)	-0.091, -0.053	0.001	0.28 - 0.43 [0.13, 0.59]
20:1 Eicosenoic	0.33 (0.016) [0.29 - 0.37]	0.19 (0.016) [0.17 - 0.21]	0.14 (0.0086)	0.11, 0.17	<0.001	0.18 - 0.27 [0.066, 0.37]

Table E-17 (continued). Comparison of Fatty Acids and Vitamin E from MON 87705 and Conventional Control (A3525) Soybean RBD Oil Processed from the 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E.[1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
RBD Oil Fatty Acid (% Total FA)						
22:0 Behenic	0.31 (0.011) [0.30 - 0.35]	0.35 (0.011) [0.33 - 0.38]	-0.034 (0.0033)	-0.044, -0.023	0.001	0.30 - 0.50 [0.11, 0.70]
24:0 Lignoceric	0.14 (0.018) [0.11 - 0.18]	0.15 (0.018) [0.11 - 0.19]	-0.0056 (0.0036)	-0.017, 0.0059	0.218	0.088 - 0.23 [0, 0.36]
Vitamin E (mg/100g FW)	11.64 (2.96) [6.37 - 17.35]	13.09 (2.96) [7.72 - 20.35]	-1.45 (0.67)	-3.59, 0.70	0.120	5.36 - 31.55 [0, 56.40]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval; ND = not detected; NC = not calculated

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

Table E-18. Comparison of Amino Acids and Moisture from MON 87705 and Conventional Control (A3525) Soybean Protein Isolate Processed from a 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E. [1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial Range [99% Tolerance Int.[2]]
Amino Acid (%DW)						
Alanine	3.73 (0.029) [3.66 - 3.81]	3.71 (0.029) [3.64 - 3.76]	0.017 (0.015)	-0.031, 0.064	0.345	3.62 - 3.90 [3.39, 4.19]
Arginine	7.84 (0.094) [7.57 - 8.08]	7.83 (0.094) [7.68 - 8.04]	0.0065 (0.11)	-0.34, 0.35	0.955	7.69 - 8.17 [7.38, 8.51]
Aspartic Acid	10.88 (0.10) [10.58 - 11.19]	10.87 (0.10) [10.68 - 11.04]	0.013 (0.14)	-0.42, 0.45	0.930	10.93 - 11.54 [10.38, 11.90]
Cystine	1.21 (0.024) [1.17 - 1.28]	1.18 (0.024) [1.12 - 1.23]	0.035 (0.034)	-0.050, 0.12	0.351	1.06 - 1.29 [0.87, 1.44]
Glutamic Acid	17.52 (0.23) [16.89 - 18.15]	17.66 (0.23) [17.25 - 18.02]	-0.14 (0.20)	-0.77, 0.50	0.540	18.24 - 20.10 [16.60, 21.24]
Glycine	3.93 (0.021) [3.87 - 3.98]	3.92 (0.021) [3.90 - 3.97]	0.0027 (0.029)	-0.090, 0.095	0.932	3.86 - 4.04 [3.71, 4.17]
Histidine	2.40 (0.019) [2.35 - 2.44]	2.39 (0.019) [2.36 - 2.44]	0.0026 (0.017)	-0.052, 0.058	0.888	2.31 - 2.55 [2.16, 2.73]

Table E-18 (continued). Comparison of Amino Acids and Moisture from MON 87705 and Conventional Control (A3525) Soybean Protein Isolate Processed from a 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E. [1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Isoleucine	4.43 (0.039) [4.35 - 4.48]	4.47 (0.039) [4.36 - 4.56]	-0.032 (0.024)	-0.11, 0.046	0.280	4.44 - 4.78 [4.14, 5.10]
Leucine	7.33 (0.037) [7.19 - 7.41]	7.33 (0.037) [7.28 - 7.38]	-0.00005 (0.039)	-0.12, 0.12	0.999	7.36 - 7.76 [7.10, 8.06]
Lysine	5.80 (0.017) [5.75 - 5.83]	5.76 (0.017) [5.74 - 5.81]	0.034 (0.015)	-0.013, 0.081	0.102	5.81 - 6.03 [5.67, 6.15]
Methionine	1.27 (0.024) [1.25 - 1.29]	1.24 (0.024) [1.15 - 1.29]	0.035 (0.030)	-0.061, 0.13	0.327	1.13 - 1.25 [0.99, 1.41]
Phenylalanine	5.05 (0.038) [4.94 - 5.15]	5.07 (0.038) [5.01 - 5.14]	-0.019 (0.046)	-0.17, 0.13	0.713	5.04 - 5.50 [4.68, 5.71]
Proline	5.02 (0.051) [4.91 - 5.11]	5.05 (0.051) [4.92 - 5.17]	-0.033 (0.049)	-0.19, 0.12	0.555	4.54 - 4.99 [4.29, 5.27]
Serine	4.96 (0.043) [4.87 - 5.06]	4.92 (0.043) [4.86 - 5.02]	0.043 (0.061)	-0.11, 0.19	0.513	4.54 - 5.15 [4.00, 5.76]
Threonine	3.37 (0.037) [3.28 - 3.43]	3.35 (0.037) [3.24 - 3.43]	0.018 (0.0085)	-0.0086, 0.045	0.118	3.22 - 3.45 [2.99, 3.62]

Table E-18 (continued). Comparison of Amino Acids and Moisture from MON 87705 and Conventional Control (A3525) Soybean Protein Isolate Processed from a 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E. [1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tryptophan	1.05 (0.016) [1.01 - 1.07]	1.04 (0.016) [0.99 - 1.07]	0.0071 (0.011)	-0.027, 0.042	0.556	0.90 - 1.06 [0.76, 1.16]
Tyrosine	3.46 (0.012) [3.43 - 3.49]	3.45 (0.012) [3.43 - 3.48]	0.0089 (0.013)	-0.032, 0.050	0.536	3.45 - 3.64 [3.25, 3.85]
Valine	4.42 (0.052) [4.34 - 4.49]	4.45 (0.052) [4.29 - 4.58]	-0.031 (0.054)	-0.20, 0.14	0.610	4.46 - 4.94 [4.04, 5.27]
Moisture (% FW)	1.91 (0.28) [1.30 - 2.96]	1.33 (0.28) [0.93 - 1.69]	0.58 (0.27)	-0.27, 1.43	0.117	1.32 - 4.71 [0, 6.54]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

Table E-19. Comparison of Phosphatides from MON 87705 and Conventional Control (A3525) Soybean Lecithin Processed from a 2007 U.S. Field Production

Analytical Component[1]	MON 87705 Mean ± S.E.[1] [Range]	A3525 Mean ± S.E. [Range]	Difference (MON 87705 minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Phosphatides (% FW)						
L-alpha-Phosphatidic Acid	1.10 (0.29) [0.35 - 1.64]	1.24 (0.29) [0.35 - 1.60]	-0.14 (0.41)	-1.14, 0.86	0.740	1.30 - 4.19 [0, 5.64]
L-alpha-Phosphatidylcholine	4.38 (1.04) [1.93 - 7.85]	7.18 (1.04) [5.75 - 9.18]	-2.80 (1.14)	-6.44, 0.83	0.091	2.88 - 7.11 [0, 11.42]
L-alpha-Phosphatidylethanolamine	2.45 (0.74) [0.65 - 4.79]	4.99 (0.74) [4.06 - 6.70]	-2.55 (0.97)	-5.62, 0.53	0.077	2.21 - 5.50 [0, 8.78]
L-alpha-Phosphatidylinositol	2.28 (0.66) [1.13 - 4.53]	4.63 (0.66) [3.99 - 6.06]	-2.36 (0.83)	-5.00, 0.29	0.066	2.19 - 5.52 [0, 8.67]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

Table E-20. Literature Ranges for Components in Soybean Meal

Tissue Component	Literature Range[1]
Proximates	**(% dw)**
Ash	5.2 – 9.1[a]
Carbohydrates	32.0 – 38.0[b]
Fat, total	0.5 -3.30[a]
Moisture (% fw)	5.58-11.7[a]
Protein	47.4 – 59.5[a]
Fiber	**(% dw)** [a]
Acid detergent fiber (ADF)	5.2 – 6.7
Neutral detergent fiber (NDF)	7.4 – 12.2
Amino Acids	**(%dw)**[a]
Alanine	2.18 – 2.59
Arginine	3.29 – 4.49
Aspartic acid	5.18 – 6.83
Cystine/Cysteine	0.6 – 0.92
Glutamic acid	8.05 – 11.21
Glycine	2.02 – 2.40
Histidine	1.32 – 1.63
Isoleucine	2.11 – 2.74
Leucine	3.62 – 4.72
Lysine	2.97 – 3.69
Methionine	0.5 – 0.9
Phenylalanine	2.39 – 3.19
Proline	2.32 – 3.05
Serine	1.97 – 3.3
Threonine	0.80 – 2.24
Tryptophan	0.60 – 2.08
Tyrosine	1.68 – 2.17
Valine	2.29 – 2.92
Anti-Nutrients	
Trypsin Inhibitors (TIU/mg dw)	3.8 – 17.9[a]
Phytic Acid (% dw)	1.3 – 4.1 [a]

[1]Literature range references: [a]Lundry, et al., 2008. [b]Padgette et al., 1996.

Table E-21. Literature Ranges for Components in Soybean Protein Isolate

Tissue Component	Literature Range or Value[1]
Proximates	
Moisture (% fw)	3.9 – 7.0
Amino Acids	**(%dw)**
Alanine	NA
Arginine	6.67
Aspartic acid	NA
Cystine/Cysteine	1.05
Glutamic acid	NA
Glycine	NA
Histidine	2.3
Isoleucine	4.25
Leucine	6.78
Lysine	5.33
Methionine	1.13
Phenylalanine	4.59
Proline	NA
Serine	NA
Threonine	3.14
Tryptophan	1.12
Tyrosine	NA
Valine	4.1

[1]Literature range or value reference: Lundry, et. al., 2008.

Table E-22. Literature Ranges for Components in Soybean Lecithin

Tissue Component	Literature Range[1]
Phosphatides (%fw)	
α-Phosphatidic Acid	0.2 – 14.0
α-Phosphatidylcholine	12.0 – 46.0
α-Phosphatidylethanolamine	8.0 – 34.0
α-Phosphatidylinositol	1.7 – 21.0

Literature range reference: [1] Lundry, et. al., 2008.

Table E-23. Literature Ranges for Components in Soybean Oil

Tissue Component	Literature Range[1]
Fatty Acids (FA)	
14:0 Myristic	ND – 0.2[a]
16:0 Palmitic	7 - 12[b]
16:1 Palmitoleic	≤ 0.2[b]
17:0 Margaric [Heptadecanoic]	ND – 0.1[a]
17:1 9c Heptadecenoic	ND – 0.1[a]
18:0 Stearic	2 – 5[b]
18:1 Oleic	19 – 34[b]
18:2 Linoleic	48 – 60[b]
18:3 Linolenic	2 -10[b]
20:0 Arachidic	0.1 – 0.6[a]
20:1 Eicosenoic	ND – 0.5[a]
20:2 Eicosadienoic	ND – 0.1[a]
22:0 Behenic	ND – 0.7[a]
Vitamins	**mg/100g fw**
Vitamin E	0.9 – 35.2[a]

[1]Literature range references: [a]Codex, 2005 (% Total FA). [b]Lundry, et al., 2008, (% FW). ND = not detected.

Appendix E References

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Appendix F. Materials and Methods for Seed Dormancy and Germination Analyses of MON 87705

F.1 Materials

MON 87705, a conventional soybean control (A3525), and commercial soybean reference variety starting seed were produced in Jefferson County, IA; Boone County, IN; and Macon County, MO in 2007.

F.2 Characterization of the Materials

For the MON 87705, conventional soybean control, and commercial soybean reference variety starting seed, the presence or absence of MON 87705 was verified by event-specific polymerase chain reaction (PCR) analyses. The results of these analyses confirmed the presence of MON 87705 in the MON 87705 starting seed and the absence of MON 87705 in the control and reference variety seed.

F.3 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, a seed trade association (AOSA, 2000; AOSA, 2006; AOSA, 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 ^{o}C or alternating temperatures of approximately 10/20, 10/30, or 20/30 ^{o}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 87705, 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 ^{o}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; AOSA, 2007). Each rolled germination towel in the additional temperature regimes (i.e., 10, 20, 30, 10/20 and 10/30 ^{o}C) was assessed periodically during the study for germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed.

F.4 Statistical Analysis

Statistical analyses were performed by the Monsanto Statistics Technology Center. Analysis of variance was conducted according to three randomized complete block designs 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 87705 to the conventional soybean control within each seed 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 $\alpha = 0.05$. The MON 87705 substance was not statistically compared to the reference substances, nor were comparisons made across temperature regimes. The minimum and maximum mean values (reference range) were determined from the reference substances at each site.

F.5 Individual Site Seed Dormancy and Germination Analysis

There were no statistically significant differences detected between MON 87705 and the control for any of the measured characteristics (i.e., percent germinated, viable hard, or viable firm swollen seed) in any temperature regime for seed produced at the IN site. Seven statistically significant differences were detected between MON 87705 and the control (Table F-2) for seed produced at the IA and MO sites. MON 87705 had lower percent normal germination than the control at 20/30 ºC for seed produced at the IA site (89.3 vs. 94.0%). At 30 °C, MON 87705 had lower percent germination than the control for seed produced at the IA site (82.8 vs. 95.5%) and higher percent germination than the control for seed produced at the MO site (100.0 vs. 96.3%). MON 87705 had lower percent viable hard seed than the control at 20° C for seed produced at the MO site (0.0 vs. 0.8%). Percent dead seed was higher for MON 87705 than the control at 30 ºC (17.3 vs. 4.5%) and 20/30 ºC (6.0 vs. 2.5%) for seed produced at the IA site and lower for MON 87705 than the control at 30 ºC for seed produced at the MO site (0.0 vs. 3.8%).

Table F-1. Starting Seed of MON 87705, Control and Commercial Reference Soybean Varieties Used in Dormancy Assessment

Production Site	Substance Name	Substance Type	Phenotype[1]	Sample ID Number
IA	MON 87705	Test	Modified oil profile	RPN07273-001
IA	A3525	Control	Conventional	RPN07273-002
IA	Hoegemeyer 333	Reference	Conventional	RPN07273-005
IA	Stewart 3454	Reference	Conventional	RPN07273-006
IA	Stine 3600-0	Reference	Conventional	RPN07273-007
IA	Lewis 372	Reference	Conventional	RPN07273-008
IN	MON 87705	Test	Modified oil profile	RPN07273-009
IN	A3525	Control	Conventional	RPN07273-010
IN	Midland 363	Reference	Conventional	RPN07273-013
IN	NK S33A8	Reference	Glyphosate tolerant[2]	RPN07273-014
IN	Asgrow AG3505	Reference	Glyphosate tolerant[2]	RPN07273-015
IN	FS 3591	Reference	Conventional	RPN07273-016
MO	MON 87705	Test	Modified oil profile	RPN07273-017
MO	A3525	Control	Conventional	RPN07273-018
MO	Hoegemeyer 333	Reference	Conventional	RPN07273-021
MO	Stewart 3454	Reference	Conventional	RPN07273-022
MO	Stine 3600-0	Reference	Conventional	RPN07273-023
MO	Lewis 372	Reference	Conventional	RPN07273-024

[1] MON 87705 possesses the improved fatty acid profile trait; the control and reference soybean varieties do not possess the trait.
[2] Glyphosate tolerant = Commercially available Roundup Ready soybean 40-3-2 variety.

Table F-2. Comparison of MON 87705 to the Control for Dormancy and Germination Characteristics

Temp. Regime	Category	IA[1] Mean % (S.E.)[2]			IN[1] Mean % (S.E.)[2]			MO[1] Mean % (S.E.)[2]		
		MON 87705	Control	Reference Range[3]	MON 87705	Control	Reference Range[3]	MON 87705	Control	Reference Range[3]
10 °C	Germinated	96.8 (1.5)	98.0 (1.2)	99.3 – 100.0	97.5 (1.7)	98.8 (0.5)	98.5 – 99.3	98.8 (0.6)	98.5 (0.6)	96.5 – 99.3
	Viable Hard	0.0 (0.0)	0.3 (0.3)	0.0 – 0.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.3 (0.3)	0.5 (0.3)	0.5 – 1.5
	Dead	3.0 (1.6)	1.8 (1.0)	0.0 – 0.8	2.3 (1.7)	0.8 (0.3)	0.8 – 1.3	1.0 (0.7)	0.8 (0.5)	0.3 – 1.8
	Viable Firm Swollen	0.3 (0.3)	0.0 (0.0)	0.0 – 0.0	0.3 (0.3)	0.5 (0.3)	0.0 – 0.5	0.0 (0.0)	0.3 (0.3)	0.0 – 0.3
20 °C	Germinated	97.5 (1.3)	98.5 (0.6)	98.8 – 99.5	99.8 (0.3)	99.8 (0.3)	99.5 – 99.8	100.0 (0.0)	98.3 (0.5)	95.0 – 98.8
	Viable Hard	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0)	0.3 (0.3)	0.0 – 0.0	0.0 (0.0) *	0.8 (0.3)	0.0 – 0.5
	Dead	2.5 (1.3)	1.5 (0.6)	0.5 – 1.3	0.3 (0.3)	0.0 (0.0)	0.3 – 0.5	0.0 (0.0)	1.0 (0.6)	0.8 – 5.0
	Viable Firm Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0
30 °C	Germinated	82.8 (3.1)*	95.5 (1.3)	97.5 – 98.5	95.5 (1.2)	94.8 (0.9)	96.0 – 99.5	100.0 (0.0) *	96.3 (0.9)	91.0 – 98.5
	Viable Hard	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.0 (0.0)	0.0 (0.0)	0.0 – 0.5
	Dead	17.3 (3.1) *	4.5 (1.3)	1.5 – 2.5	4.5 (1.2)	5.3 (0.9)	0.3 – 4.0	0.0 (0.0) *	3.8 (0.9)	1.3 – 8.5
	Viable Firm Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0

Table F-2 (cont.). Comparison of MON 87705 to the Control for Dormancy and Germination Characteristics

Temp. Regime	Category	IA[1] Mean % (S.E.)[2]			IN[1] Mean % (S.E.)[2]			MO[1] Mean % (S.E.)[2]		
		MON 87705	Control	Reference Range[3]	MON 87705	Control	Reference Range[3]	MON 87705	Control	Reference Range[2]
10/20°C	Germinated	99.3 (0.3)	99.5 (0.5)	98.8 – 99.5	99.5 (0.3)	99.8 (0.3)	99.3 – 100.0	99.3 (0.5)	99.5 (0.5)	97.8 – 99.8
	Viable Hard	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.3 (0.3)	0.3 (0.3)	0.0 – 0.5
	Dead	0.8 (0.3)	0.5 (0.5)	0.5 – 1.3	0.5 (0.3)	0.3 (0.3)	0.0 – 0.5	0.5 (0.3)	0.3 (0.3)	0.0 – 2.3
	Viable Firm Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0
10/30°C	Germinated	**95.3 (1.3)**	**97.5 (1.0)**	99.3 – 99.5	99.3 (0.5)	99.3 (0.3)	98.8 – 99.5	99.5 (0.3)	99.5 (0.3)	96.8 – 99.5
	Viable Hard	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.0 (0.0)	0.0 (0.0)	0.0 – 0.5
	Dead	4.8 (1.3)	2.5 (1.0)	0.5 – 0.8	0.8 (0.5)	0.8 (0.3)	0.5 – 1.3	0.5 (0.3)	0.5 (0.3)	0.5 – 2.8
	Viable Firm Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0
20/30°C (AOSA)	Normal Germinated	89.3 (1.3) *	94.0 (1.1)	95.8 – 97.3	96.0 (0.8)	94.5 (1.0)	57.0 – 98.5	92.0 (0.9)	92.3 (1.3)	81.0 – 95.0
	Abnormal Germinated	4.8 (1.3)	3.5 (1.4)	2.0 – 3.8	3.5 (1.0)	5.5 (1.0)	1.3 – 42.5	6.5 (0.6)	6.0 (1.1)	3.8 – 14.8
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.0 (0.0)	0.0 (0.0)	0.0 – 0.0	0.0 (0.0)	0.5 (0.3)	0.0 – 0.5
	Dead	6.0 (0.4) *	2.5 (0.5)	0.0 – 1.0	0.5 (0.3)	0.0 (0.0)	0.3 – 1.0	1.5 (0.5)	1.3 (0.3)	0.5 – 4.3
	Viable Firm Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3

* Indicates a statistically significant difference between MON 87705 and the conventional soybean control ($p < 0.05$).
† No statistical comparison could be made due to lack of variability in the data.
[1] Site codes are as follows: IA = Jefferson County, IA; IN = Boone County, IN; MO = Macon County, MO.
[2] Means based on four replicates (n = 4) of approximately 100 seeds. In some instances, the total percentage of both MON 87705 and the control did not equal exactly 100% due to numerical rounding of the means. S.E. = Standard Error.
[3] Minimum and maximum mean values determined from commercially available reference soybean varieties

Appendix F References

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AOSA. 2006. Seedling Evaluation Handbook, Contribution No. 35 to the Handbook on Seed Testing. Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA. 2007. Rules for Testing Seeds. Association of Official Seed Analysts, Lincoln, Nebraska.

Appendix G. Material, Methods and Individual Site Results from Phenotypic, Agronomic and Ecological Interactions Analyses of MON 87705

G.1 Materials

The materials for phenotypic assessments include: MON 87705, a conventional soybean control (A3525), and 17 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 87705 and control (A3525) seed were confirmed by PCR analysis prior to use.

G.2 Field Sites and Plot Design

Data were collected from field trials conducted in 2007 at 17 sites within U.S. soybean production regions (Section VIII, Table VIII-3). The 17 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 17 sites in a randomized complete block design with three replications. Each plot at the IA1, IL2, IN1, and MO2 sites consisted of eight 30 ft long rows with inter-row spacing of approximately 30 inches. 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 AR, IA2, IL1, IL3, IN2, KS, MI, MO3, MO4, NE, OH, PA, and WI sites consisted of four 20 ft long rows with inter-row spacing of approximately 30 inches. Rows # 2 and 3 were designated for the collection of phenotypic, abiotic stress response, disease damage, and arthropod damage data. Rows # 1 and 4 were used as buffer rows. The entire plot area was surrounded by an approximately 10 ft, four-row border of a commercially available soybean variety.

Table G-1. Starting Seed for Phenotypic Assessments

Substance	Substance type	Relative Maturity Group	Phenotype[1]	Monsanto Lot #	Sites[2]
MON 87705	Test	3.5	Improved Fatty Acid Profile	GLP-0702-18254-S	All sites
A3525	Control	3.5	Conventional	GLP-0702-18252-S	All sites
A3244	Reference	3.2	Conventional	GLP-0703-18458-S	All sites
Croplan HT3596STS	Reference	3.5	Conventional	GLP-0703-18386-S	AR, IL2, KS, MO3, PA
Pioneer 93M50	Reference	3.5	Roundup Ready [3]	GLP-0604-17240-S	AR, IL2, KS, MO3, PA
Channel Bio 3461	Reference	3.4	Conventional	GLP-0605-17332-S	AR, IL2, KS, MO3, PA
Pioneer 93M52	Reference	3.5	Conventional	GLP-0703-18407-S	AR, IL2, KS, MO3, PA
Hoegemeyer 333	Reference	3.2	Conventional	GLP-0703-18383-S	IA1, IL3, MI, MO4, WI
Stewart 3454	Reference	3.4	Conventional	GLP-0703-18426-S	IA1, IL3, MI, MO4, WI
Stine 3600-0	Reference	3.6	Conventional	GLP-0605-17336-S	IA1, IL3, MI, MO4, WI
Lewis 372	Reference	3.7	Conventional	GLP-0604-17261-S	IA1, IL3, MI, MO4, WI
Midland 363	Reference	3.3	Conventional	GLP-0703-18384-S	IA2, IN1, NE
NK S33A8	Reference	3.3	Roundup Ready [3]	GLP-0604-17243-S	IA2, IN1, NE
Asgrow AG3505	Reference	3.5	Roundup Ready [3]	GLP-0604-17250-S	IA2, IN1, NE
FS 3591	Reference	3.5	Conventional	GLP-0703-18387-S	IA2, IN1, NE
Stine 3300-0	Reference	3.3	Conventional	GLP-0605-17335-S	IL1, IN2, MO2, OH
DeKalb DKB34-51	Reference	3.4	Roundup Ready [3]	GLP-0604-17254-S	IL1, IN2, MO2, OH
Garst 3585N	Reference	3.5	Conventional	GLP-0703-18388-S	IL1, IN2, MO2, OH
Crows C37003N	Reference	3.7	Conventional	GLP-0703-18390-S	IL1, IN2, MO2, OH

[1] MON 87705 expresses the improved fatty acid profile trait; whereas the conventional soybean control and reference varieties do not express the improved fatty acid profile trait.

[2] MON 87705, the control, and reference material A3244 were planted at all field sites; the remaining reference varieties were site-specific. Site codes are as follows: AR = Jackson County, AR; IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Clinton County, IL; IL2 = Stark County, IL, IL3 = Warren County, IL; IN1 = Boone County, IN; IN2 = Parke County, IN; KS = Pawnee County, KS; MI = Ottawa County, MI; MO2 = Lincoln County, MO, MO3 = St. Louis County, MO; MO4 = Macon County, MO; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA; WI = Walworth County, WI.

[3] Commercially available glyphosate-tolerant (Roundup Ready 40-3-2) soybean variety.

Table G-2. Field and Planting Information

Site[1]	Planting date (mm/dd/yr)	Seeding rate (seeds/ft)[2]	Planting depth (in)	Plot size (ft)[3]	Rows/ plot	Soil series, organic matter, pH	Cropping history	
							2006	2005
AR	05/26/2007	8	0.75	10 x 20	4	Crowley silt loam, 2.5%, 6.2	Rice	Soybean
IA1	05/23/2007	8	1.25	20 x 30	8	Tainter/Mahaska silty clay loam, 4.02%, 6.75	Corn	Corn
IA2	05/26/2007	8	1.5	10 x 20	4	Tama-Muscatine silty clay loam, 2.8%, 6.3	Milk thistle	Corn
IL1	06/04/2007	8	1.0	10 x 20	4	Silt loam, 2.65%, 6.59	Milo	Fallow
IL2	05/31/2007	8	1.5	20 x 30	8	Plano silt loam, 3.5%, 6.4	Corn	Soybean and Corn
IL3	05/23/2007	8	1.0	10 x 20	4	Sable silty clay loam, 6.0%, 5.6-7.3	Corn	Soybean
IN1	05/30/2007	8	1.5	20 x 30	8	Crosby silty clay loam, 2.4%, 6.8	Corn	Soybean
IN2	06/08/2007	8	1.25	10 x 20	4	Reesville silt loam, 1.4%, 5.8	Corn	Corn
KS	05/22/2007	9	1.0	10 x 20	4	Farnum loam, 2.6%, 7.6	Wheat	Alfalfa
MI	05/23/2007	8	1.5	10 x 20	4	Nester loam, 2.1%, 6.5	Corn	Soybean
MO2	05/30/2007	8	1.5	20 x 30	8	Crider silt loam, 2.3%, 6.7	Corn	Soybean
MO3	05/25/2007	8	1.5	10 x 20	4	Kennebec silt loam, 2.6%, 6.3	Corn	Soybean
MO4	06/07/2007	8	1.0	10 x 20	4	Gorin silt loam, 4.2%, 5.8	Fescue	Fescue
NE	05/21/2007	8	1.0	10 x 20	4	Hastings silt loam, 3.0%, 6.6	Soybean	Soybean
OH	05/22/2007	8	1.0	10 x 20	4	Crosby silt loam, 1.8%, 6.6	Corn	Soybean
PA	06/09/2007	8	1.5	10 x 20	4	Philo/Atkins silt loam, 2.4%, 5.8	Tomato	Corn
WI	05/29/2007	8	1.0	10 x 20	4	Radford silt loam, not available, not available	Wheat	Soybean

[1] Site codes are as follows: AR = Jackson County, AR; IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Clinton County, IL; IL2 = Stark County, IL, IL3 = Warren County, IL; IN1 = Boone County, IN; IN2 = Parke County, IN; KS = Pawnee County, KS; MI = Ottawa County, MI; MO2 = Lincoln County, MO, MO3 = St. Louis County, MO; MO4 = Macon County, MO; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA; WI = Walworth County, WI.

[2] The KS site planted the production at nine seeds per foot as specified in the study protocol.

[3] Width × length.

G.3 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.

G.4 Phenotypic Observations

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

G.5 Ecological Observations

Ecological interactions (i.e., interactions between the crop plants and their receiving environment) were used to characterize MON 87705 by evaluating plant response to abiotic stressors, disease damage, arthropod damage, and pest and beneficial arthropod abundance in the plots using the following methods:

G.6 Abiotic Stress Response, Disease Damage, and Arthropod Damage

MON 87705 and the conventional soybean control were evaluated at each of 17 sites (AR, IA1, IA2, IL1, IL2, IL3, IN1, IN2, KS, MI, MO2, MO3, MO4, NE, OH, PA, and WI) for differences in plant response to abiotic stressors, disease damage, and arthropod damage. Three abiotic stressors, three diseases, and three arthropod pests were evaluated four times during the growing season at the following intervals:

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

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

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

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

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

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

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

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

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

For each abiotic stress response, disease damage, and arthropod damage observation at a site, the range of injury severity ratings observed across all three replications for each of MON 87705, 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.

G.7 Arthropod Abundance

Pest and beneficial arthropods were collected at the IA1, IL2, IN1, and MO2 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 subsamples were collected in this way from each plot. Specifically, two subsamples were collected from Rows # 5 and 6 of each plot (subsamples 1 and 3) and two subsamples were collected from Rows # 6 and 7 of each plot (subsamples 2 and 4). The subsamples collected from the same row were at least 10 ft apart and at least 3 ft from the edge of each plot. The four subsamples were combined into one pre-labelled container and placed on dry ice. The samples were then sent overnight to the Monsanto Regulatory Environmental Science Center for arthropod identification and enumeration.

A maximum of the six most abundant pest and six most abundant beneficial arthropods were determined for each collection interval from each individual site. These specific arthropods were then enumerated across all samples from a given collection interval at each individual 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.

G.8 Ecological Interactions Evaluation Criteria

For the assessments of abiotic stress response, disease damage, and arthropod damage, MON 87705 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 87705 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 commercial soybean varieties. Pest and beneficial arthropod abundance data were quantitatively evaluated and subjected to statistical analysis, as appropriate.

G.9 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.

G.10 Statistical Analysis

Analysis of variance was conducted according to a randomized complete block design using SAS (Version 9.2). The level of significance was $\alpha = 0.05$. MON 87705 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. 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 87705 and reference soybean varieties. The reference range for each measured phenotypic characteristic was determined from the minimum and maximum mean values collected from the 17 reference soybean varieties planted among the sites. The reference range for the plant response to abiotic stressor, disease, arthropod damage, and abundance of each arthropod evaluated from a given collection and site was determined from the minimum and maximum mean values collected from the reference varieties at the site. Thus, reference ranges for abiotic stressor, disease susceptibility, arthropod damage, and arthropod abundance were specific to each collection/observation interval and site.

G.11 Individual Field Site Plant Growth and Development Results and Discussion

In the individual-site analysis, lack of variability in the data precluded statistical comparisons between MON 87705 and the control for seedling vigor at the IL1, IL3, MO4, and PA sites; days to 50% flowering at the IA2, IN1, NE, and WI sites; plant lodging ratings at the IL1, IN1, MO2, MO3, and NE sites; pod shattering at the IL1, IL2, IL3, IN2, MI, MO2, MO3, NE, OH, PA, and WI sites; and test weight at the WI site. In each of these cases, however, the mean for MON 87705 and the mean for the control were the same value or nearly the same value (Table G-3).

In the individual-site analysis, a total of 30 statistically significant differences were detected out of 161 comparisons between MON 87705 and the conventional soybean control (Table G-3). These differences were distributed among nine of the 13 phenotypic characteristics. Early stand count was lower for MON 87705 than the control at the AR site (101.7 vs. 155.3 plants/plot), the IL1 site (253.3 vs. 271.0 plants/plot), the IL2 site (240.6 vs. 284.6 plants/plot), the OH site (112.0 vs. 159.0 plants/plot), and the PA site (262.3 vs. 288.0 plants/plot). MON 87705 flowered later than the control at the IL2 site (208.0 vs. 205.3 days after 1 Jan. 2007), the IN2 site (204.7 vs. 203.0 days after 1 Jan. 2007), the MO2 site (193.7 vs. 191.0 days after 1 Jan. 2007), the MO4 site (204.3 vs. 201.3 days after 1 Jan. 2007), and the PA site (209.0 vs. 207.3 days after 1 Jan. 2007). Plants of MON 87705 were taller than the control at the IN2 site (35.4 vs. 33.6 inches) but shorter than the control at the IA1 (40.3 vs. 42.9 inches), the MO3 (29.6 vs. 32.0 inches), and the PA (28.6 vs. 32.0 inches) sites. MON 87705 had more lodging than the control at the IN2 site (4.0 vs. 1.7 rating). Final stand count was lower for MON 87705 than the control at the AR (98.0 vs. 153.7 plants/plot), the IA1 (172.8 vs. 213.0 plants/plot), the IL1 (254.3 vs. 276.0 plants/plot), the IL2 (206.6 vs. 248.1 plants/plot), the IN1 (226.3 vs. 255.7 plants/plot), and the OH (116.7 vs. 156.7 plants/plot) sites. Seed moisture was lower for MON 87705 than the control at the KS (11.8 vs. 13.4%) and the MO3 (12.0 vs. 14.4%) sites. The weight of 100 seeds was lower for MON 87705 than the control at the IA2 (13.8 vs. 14.4 g), MI (19.6 vs. 21.3 g), and MO4 (13.3 vs. 14.5 g) sites. Test weight was greater for MON 87705 than the control at the IN1 site (50.1 vs. 42.1 lb/bu), but less than the control at the MO4 site (50.7 vs. 53.8 lb/bu). Yield was lower for MON 87705 than the control at the IL2 (43.5 vs. 51.2 bu/ac) and MO3 (21.7 vs. 31.8 bu/ac) sites. Considering that the statistical differences for plant height, lodging, seed moisture, test weight, and yield that were detected in the individual-site analyses 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 87705 compared to the conventional soybean control. While some statistical differences were detected in the combined-site analysis, the assessed phenotypic values of MON 87705 were within the range of values expected for commercial soybean.

Table G-3. Phenotypic Comparison of MON 87705 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 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)	MON 87705	Control	MON 87705	Control
AR	101.7* (13.9)	155.3 (17.5)	6.0 (0.6)	5.3 (0.3)	180.0 (1.2)	178.0 (1.2)	Purple	Purple	Hairy	Hairy
IA1	261.7 (8.9)	282.6 (5.4)	3.0 (0.6)	2.7 (0.7)	192.7 (0.3)	191.7 (0.3)	Purple	Purple	Hairy	Hairy
IA2	266.7 (5.6)	282.3 (2.3)	3.3 (0.3)	3.0 (0.0)	197.0‡ (0.0)	197.0 (0.0)	Purple	Purple	N/A	N/A
IL1	253.3* (2.7)	271.0 (5.1)	1.0‡ (0.0)	1.0 (0.0)	201.0 (0.0)	201.3 (0.3)	Purple	Purple	Hairy	Hairy
IL2	240.6* (8.5)	284.6 (3.5)	1.0 (0.0)	1.0 (0.0)	208.0* (1.0)	205.3 (0.3)	Purple	Purple	Hairy	Hairy
IL3	283.3 (11.0)	302.0 (9.9)	2.0‡ (0.0)	2.0 (0.0)	190.0 (1.0)	188.3 (0.7)	Purple	Purple	Hairy	Hairy
IN1	222.8 (15.1)	248.6 (5.8)	2.3 (0.3)	3.0 (1.0)	207.0‡ (0.0)	207.0 (0.0)	Purple	Purple	Hairy	Hairy
IN2	257.3 (6.9)	259.7 (7.2)	3.7 (0.3)	3.3 (0.7)	204.7* (0.3)	203.0 (0.0)	Purple	Purple	Hairy	Hairy
KS	158.3 (15.9)	180.7 (8.7)	4.0 (0.0)	4.0 (0.0)	191.0 (0.6)	191.0 (0.0)	Purple	Purple	Hairy	Hairy
MI	320.3 (1.5)	303.7 (14.3)	3.3 (0.3)	4.0 (0.6)	198.7 (0.7)	198.0 (0.0)	Purple	Purple	Hairy	Hairy
MO2	288.6 (8.0)	299.5 (6.7)	5.3 (0.3)	5.0 (0.0)	193.7* (0.3)	191.0 (0.6)	Purple	Purple	Hairy	Hairy
MO3	237.3 (17.5)	280.0 (5.8)	4.7 (0.7)	3.3 (0.3)	191.7 (1.3)	190.0 (1.5)	Purple	Purple	Hairy	Hairy
MO4	201.3 (12.8)	218.3 (6.6)	2.0‡ (0.0)	2.0 (0.0)	204.3* (0.7)	201.3 (0.3)	Purple	Purple	N/A	N/A
NE	308.0 (6.1)	289.3 (13.1)	3.0 (0.0)	3.0 (0.0)	192.0‡ (0.0)	192.0 (0.0)	Purple	Purple	Hairy	Hairy
OH	112.0* (11.0)	159.0 (10.2)	3.7 (0.9)	3.3 (0.7)	199.3 (0.7)	197.3 (0.9)	Purple	Purple	Hairy	Hairy
PA	262.3* (6.6)	288.0 (4.2)	2.0‡ (0.0)	2.0 (0.0)	209.0* (0.0)	207.3 (0.3)	Purple	Purple	Hairy	Hairy
WI	221.0 (20.0)	249.7 (2.9)	1.3 (0.3)	1.7 (0.7)	207.0‡ (0.0)	207.0 (0.0)	Purple	Purple	Hairy	Hairy

* Indicates a statistically significant difference between MON 87705 and the conventional soybean control ($p < 0.05$).
‡ Indicates a lack of variability in the data which precluded statistical analysis.
N/A = Data not available or excluded from the data analysis.
[1] Site codes are as follows: AR = Jackson County, AR; IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Clinton County, IL; IL2 = Stark County, IL, IL3 = Warren County, IL; IN1 = Boone County, IN; IN2 = Parke County, IN; KS = Pawnee County, KS; MI = Ottawa County, MI; MO2 = Lincoln County, MO, MO3 = St. Louis County, MO; MO4 = Macon County, MO; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA; WI = Walworth County, WI.
[2] Flower color and plant pubescence data were categorical and were not statistically analyzed.
S.E. = standard error. Means based on n = 3.

Table G-3 (cont.). Phenotypic Comparison of MON 87705 to the Conventional Soybean Control within Each Site

	Phenotypic Characteristic (units)									
	Plant height (in)		Lodging (0-9 scale)		Pod Shattering (0-9 scale)		Final stand count (#/plot)		Seed moisture (%)	
Site[1]	MON 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)
AR	25.0 (0.6)	24.1 (0.5)	2.3 (0.3)	1.0 (0.6)	0.7 (0.3)	0.3 (0.3)	98.0* (12.1)	153.7 (16.5)	10.5 (0.6)	9.6 (0.1)
IA1	40.3* (0.1)	42.9 (0.6)	5.0 (0.6)	5.0 (0.6)	0.0 (0.0)	0.3 (0.3)	172.8* (12.5)	213.0 (8.2)	10.6 (0.1)	10.7 (0.0)
IA2	42.7 (1.1)	44.3 (1.7)	4.3 (0.3)	4.3 (0.3)	0.7 (0.3)	0.7 (0.3)	238.3 (11.3)	257.7 (8.4)	12.4 (0.4)	12.3 (0.1)
IL1	24.7 (0.3)	25.1 (0.4)	0.0‡ (0.0)	0.0 (0.0)	0.0‡ (0.0)	0.0 (0.0)	254.3* (1.8)	276.0 (2.0)	9.3 (0.1)	8.9 (0.1)
IL2	41.3 (0.9)	43.1 (0.5)	3.7 (0.7)	3.7 (0.3)	0.0‡ (0.0)	0.0 (0.0)	206.6* (8.9)	248.1 (9.7)	13.2 (0.2)	12.8 (0.6)
IL3	42.8 (2.3)	43.5 (0.6)	2.3 (0.3)	2.3 (0.3)	0.0‡ (0.0)	0.0 (0.0)	264.7 (18.0)	293.3 (12.7)	9.7 (0.4)	9.5 (0.6)
IN1	33.0 (2.0)	33.3 (0.6)	0.0‡ (0.0)	0.0 (0.0)	0.3 (0.3)	0.0 (0.0)	226.3* (19.3)	255.7 (11.2)	13.8 (0.2)	13.9 (0.1)
IN2	35.4* (1.0)	33.6 (0.6)	4.0* (0.6)	1.7 (0.3)	0.0‡ (0.0)	0.0 (0.0)	231.7 (19.6)	236.0 (6.4)	13.9 (0.5)	13.9 (0.5)
KS	31.2 (0.6)	31.1 (0.1)	0.3 (0.3)	1.0 (0.0)	0.0 (0.0)	0.0 (0.0)	139.3 (16.6)	157.3 (7.3)	11.8* (0.3)	13.4 (1.0)
MI	30.5 (1.3)	31.3 (2.1)	0.0 (0.0)	0.0 (0.0)	0.0‡ (0.0)	0.0 (0.0)	285.0 (3.8)	290.3 (2.9)	13.6 (0.4)	13.0 (0.1)
MO2	32.4 (0.4)	32.4 (0.9)	0.0‡ (0.0)	0.0 (0.0)	0.0‡ (0.0)	0.0 (0.0)	288.6 (7.3)	302.4 (6.6)	15.0 (0.2)	13.7 (0.4)
MO3	29.6* (0.5)	32.0 (0.4)	0.0‡ (0.0)	0.0 (0.0)	0.0‡ (0.0)	0.0 (0.0)	241.0 (17.8)	285.0 (3.6)	12.0* (0.8)	14.4 (0.2)
MO4	29.4 (0.8)	30.2 (1.2)	0.3 (0.3)	0.7 (0.3)	0.3 (0.3)	0.3 (0.3)	189.7 (12.1)	202.7 (7.2)	10.6 (0.2)	10.5 (0.0)
NE	39.3 (0.4)	39.4 (1.4)	0.0‡ (0.0)	0.0 (0.0)	0.0‡ (0.0)	0.0 (0.0)	300.0 (2.3)	286.7 (5.8)	14.1 (0.2)	14.2 (0.0)
OH	30.0 (0.6)	33.3 (1.8)	0.0 (0.0)	0.7 (0.3)	0.0‡ (0.0)	0.0 (0.0)	116.7* (7.7)	156.7 (3.4)	12.0 (0.3)	11.9 (0.2)
PA	28.6* (0.0)	32.0 (0.6)	0.3 (0.3)	0.3 (0.3)	0.0‡ (0.0)	0.0 (0.0)	253.7 (7.8)	268.7 (3.5)	15.1 (0.5)	14.7 (0.6)
WI	45.3 (0.1)	45.0 (1.4)	7.0 (0.0)	6.3 (0.7)	1.0‡ (0.0)	1.0 (0.0)	170.3 (11.6)	185.7 (10.5)	11.6 (0.0)	11.4 (0.1)

* Indicates a statistically significant difference between MON 87705 and the conventional soybean control ($p < 0.05$).

‡ Indicates a lack of variability in the data which precluded statistical analysis.

[1] Site codes are as follows: AR = Jackson County, AR; IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Clinton County, IL; IL2 = Stark County, IL, IL3 = Warren County, IL; IN1 = Boone County, IN; IN2 = Parke County, IN; KS = Pawnee County, KS; MI = Ottawa County, MI; MO2 = Lincoln County, MO, MO3 = St. Louis County, MO; MO4 = Macon County, MO; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA; WI = Walworth County, WI.

- Data not available or excluded from the data analysis.

S.E. = standard error. Means based on n = 3.

Table G-3 (cont.). Phenotypic Comparison of MON 87705 to the Conventional Soybean Control within Each Site

	Phenotypic Characteristic (units)					
	100 seed weight (g)		Test weight (lb/bu)		Yield (bu/ac)	
Site[1]	MON 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)	MON 87705 (S.E.)	Control (S.E.)
AR	15.1 (0.1)	16.0 (0.2)	53.4 (0.1)	53.3 (0.7)	40.8 (8.9)	36.1 (7.5)
IA1	23.8 (0.0)	24.3 (0.2)	53.1 (1.7)	50.9 (1.2)	69.0 (3.8)	72.0 (2.0)
IA2	13.8* (0.1)	14.4 (0.2)	55.2 (0.2)	55.0 (0.0)	75.6 (8.5)	68.1 (1.0)
IL1	13.7 (0.9)	15.2 (0.3)	57.0 (1.0)	56.0 (0.6)	24.4 (4.4)	24.5 (2.2)
IL2	13.5 (0.1)	13.7 (0.6)	53.3 (0.9)	52.3 (0.3)	43.5* (0.5)	51.2 (2.3)
IL3	14.1 (0.3)	14.0 (0.4)	53.7 (0.8)	55.6 (0.9)	77.1 (3.5)	78.8 (5.4)
IN1	15.3 (0.3)	15.9 (0.2)	50.1* (1.6)	42.1 (0.6)	56.2 (1.7)	66.0 (5.0)
IN2	16.5 (0.1)	16.7 (0.5)	46.4 (0.6)	46.3 (0.1)	71.9 (0.9)	68.1 (3.9)
KS	13.8 (0.1)	14.1 (0.2)	56.3 (0.7)	56.0 (0.7)	65.9 (6.1)	63.6 (2.8)
MI	19.6* (0.2)	21.3 (0.6)	56.7 (0.3)	57.6 (0.2)	54.6 (1.3)	52.1 (1.3)
MO2	14.8 (0.1)	14.9 (0.2)	59.7 (0.3)	60.0 (0.5)	31.4 (1.5)	28.9 (6.9)
MO3	14.9 (0.5)	13.9 (0.6)	59.7 (0.5)	58.7 (0.2)	21.7* (0.8)	31.8 (1.1)
MO4	13.3* (0.3)	14.5 (0.4)	50.7* (1.2)	53.8 (0.4)	63.2 (4.0)	58.2 (1.9)
NE	15.0 (0.1)	14.9 (0.1)	59.0 (0.1)	59.0 (0.0)	66.1 (2.8)	67.8 (3.7)
OH	16.0 (0.4)	16.9 (0.1)	47.4 (0.6)	48.7 (1.0)	52.0 (4.3)	50.2 (1.6)
PA	16.3 (0.9)	16.7 (0.3)	55.8 (0.6)	55.8 (0.8)	54.6 (2.9)	61.0 (1.5)
WI	N/A	N/A	56.0‡ (0.0)	56.0 (0.0)	55.6 (1.9)	52.8 (1.0)

* Indicates a statistically significant difference between MON 87705 and the conventional soybean control ($p < 0.05$).
‡ Indicates a lack of variability in the data which precluded statistical analysis.
N/A = Data not available or excluded from the data analysis.
[1] Site codes are as follows: AR = Jackson County, AR; IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Clinton County, IL; IL2 = Stark County, IL, IL3 = Warren County, IL; IN1 = Boone County, IN; IN2 = Parke County, IN; KS = Pawnee County, KS; MI = Ottawa County, MI; MO2 = Lincoln County, MO, MO3 = St. Louis County, MO; MO4 = Macon County, MO; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA; WI = Walworth County, WI.
S.E. = standard error. Means based on n = 3.

Table G-4. Growth Stage Monitoring of MON 87705, 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**
AR		06/20/2007	07/09/2007	07/27/2007	08/13/2007	09/04/2007	09/25/2007	—	—	—
	MON 87705	V2-V3	V8-V9	R4-R5	R5-R6	R7	R8	—	—	—
	Control	V3	V8-V9	R4-R5	R6	R7	R8	—	—	—
	References	V2-V3	V7-V9	R4-R5	R5-R6	R7	R8	—	—	—
IA1		06/18/2007	07/09/2007	07/30/2007	08/20/2007	09/11/2007	10/02/2007	—	—	—
	MON 87705	V3	V8	R3	R5	R6-R7	R8	—	—	—
	Control	V3	V8	R3	R5	R6-R7	R8	—	—	—
	References	V3	V8	R3	R5	R6-R7	R8	—	—	—
IA2		06/20/2007	07/10/2007	07/31/2007	08/17/2007	09/05/2007	09/23/2007	10/11/2007	—	—
	MON 87705	V2	V6	R3-R4	R5	R6	R7	R8	—	—
	Control	V2	V7	R3	R5	R6	R7	R8	—	—
	References	V2	V6-V7	R3-R4	R5	R6	R7	R8	—	—
IL1		06/20/2007	07/19/2007	08/06/2007	08/27/2007	09/10/2007	09/27/2007	—	—	—
	MON 87705	V2	R1	R3	R6	R6	R8	—	—	—
	Control	V2-V3	R1	R3	R6	R6-R7	R8	—	—	—
	References	V2-V3	R1	R3	R6	R6	R7-R8	—	—	—
IL2		06/27/2007	07/11/2007	07/24/2007	08/08/2007	08/27/2007	09/17/2007	10/08/2007	—	—
	MON 87705	V2	V3-V4	R1-R2	R2-R3	R5	R7	R7-R8	—	—
	Control	V2	V3-V4	R1-R2	R3-R4	R5	R7	R7-R8	—	—
	References	V2	V3-V4	R1-R2	R2-R4	R5	R7	R7-R8	—	—

[1] Site codes are as follows: AR = Jackson County, AR; IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Clinton County, IL2 = Stark County, IL.
[2] Obs. = Observation number; dates in month/day/year format.
- Indicates information not available.

Table G-4 (continued). Growth Stage Monitoring of MON 87705, 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**
IL3		06/05/2007	06/20/2007	07/11/2007	07/27/2007	08/10/2007	08/30/2007	09/18/2007	10/04/2007	—
	MON 87705	VE	V3	R1-R2	R3	R5	R6	R7	R8	—
	Control	VE	V3	R2	R3	R5	R6	R7	R8	—
	References	VE	V3	R1-R2	R3	R5	R6	R7	R8	—
IN1		06/26/2007	07/16/2007	07/25/2007	08/17/2007	09/12/2007	10/17/2007	—	—	—
	MON 87705	V2-V3	R1	R1-R2	R3-R4	R6	R8	—	—	—
	Control	V2-V3	R1	R1-R2	R3-R4	R6	R8	—	—	—
	References	V2-V3	R1	R1-R2	R3-R4	R6-R7	R8	—	—	—
IN2		07/02/2007	07/27/2007	08/01/2007	08/16/2007	09/12/2007	09/28/2007	10/19/2007	—	—
	MON 87705	V2	R1	R2-R4	R5	R6	R8	R8	—	—
	Control	V2	R1	R2-R4	R5	R6	R8	R8	—	—
	References	V2	R1	R2-R3	R5	R6	R8	R8	—	—
KS		06/12/2007	06/29/2007	07/13/2007	08/01/2007	08/13/2007	08/24/2007	09/05/2007	10/05/2007	—
	MON 87705	V3	V8	R2	R5	R5	R6	R7	R8	—
	Control	V3	V8	R2	R5	R5	R6	R7	R8	—
	References	V3	V8	R2	R5	R5	R6	R7	R8	—
MI		06/20/2007	07/05/2007	07/18/2007	08/01/2007	08/14/2007	08/29/2007	09/12/2007	09/26/2007	10/09/2007
	MON 87705	V2	V5	R1-R2	R3	R5	R5	R6	R7	R8
	Control	V2-V3	V5	R2	R3	R5	R5	R6	R7	R8
	References	V2-V3	V5-V6	R1-R2	R2-R3	R5	R5	R6	R7	R8

[1] Site codes are as follows: IL3 = Warren County, IL; IN1 = Boone County, IN; IN2 = Parke County, IN; KS = Pawnee County, KS; MI = Ottawa County, MI.
[2] Obs. = Observation number; dates in month/day/year format.
- Indicates information not available.

Table G-4 (continued). Growth Stage Monitoring of MON 87705, 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**
MO2		06/26/2007	07/13/2007	07/25/2007	08/05/207	08/26/2007	09/17/2007	—	—	—
	MON 87705	V2-V3	V6-V8	R3-R4	R5-R6	R6	R8	—	—	—
	Control	V2-V3	V7-V8	R3-R4	R5	R6	R8	—	—	—
	References	V2-V3	V7-V9	R3-R4	R5-R6	R6	R7-R8	—	—	—
MO3		06/06/2007	06/21/2007	07/09/2007	07/24/2007	08/05/2007	08/26/2007	09/17/2007	—	—
	MON 87705	VC	V2	V7-V8	R4	R5	R6	R8	—	—
	Control	VC	V2	V7-V8	R4	R5	R6	R8	—	—
	References	VC	V2	V7-V8	R4	R5-R6	R6-R7	R8	—	—
MO4		06/29/2007	07/13/2007	08/01/2007	08/14/2007	09/11/2007	10/08/2007	—	—	—
	MON 87705	V2-V3	V6-V7	R3	R5	R6	R8	—	—	—
	Control	V3	V6-V7	R3	R5	R6	R8	—	—	—
	References	V2-V3	V6-V7	R2-R3	R5	R6	R8	—	—	—
NE		06/13/2007	07/02/2007	07/23/2007	08/13/2007	09/05/2007	09/25/2007	—	—	—
	MON 87705	V2	V6	R3	R5	R6	R7	—	—	—
	Control	V2	V6	R3	R5	R6	R7	—	—	—
	References	V2	V6	R3	R5	R6	R7	—	—	—
OH		06/15/2007	07/02/2007	07/20/2007	08/14/2007	09/04/2007	09/25/2007	—	—	—
	MON 87705	V1-V2	V3-V4	R1-R2	R3-R4	R6	R8	—	—	—
	Control	V1-V2	V3-V4	R1-R2	R3-R4	R6	R8	—	—	—
	References	V1-V2	V3-V4	R1-R2	R3-R4	R6	R8	—	—	—

[1] Site codes are as follows: MO2 = Lincoln County, MO, MO3 = St. Louis County, MO; MO4 = Macon County, MO; NE = York County, NE; OH = Fayette County, OH.
[2] Obs. = Observation number; dates in month/day/year format
- Indicates information not available.

Table G-4 (continued). Growth Stage Monitoring of MON 87705, 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**
PA		06/29/2007	07/13/2007	07/27/2007	08/13/2007	08/31/2007	09/19/2007	10/10/2007	—	—
	MON 87705	V1	V4	V7	R4	R5-R6	R6	R8	—	—
	Control	V1	V4	V7-V8	R4	R5-R6	R6	R8	—	—
	References	V1	V4	V7-V8	R3-R4	R5-R6	R6	R8	—	—
WI		06/20/2007	07/11/2007	08/16/2007	08/31/2007	09/27/2007	10/24/2007	—	—	—
	MON 87705	V2	V6	R3-R4	R5	R6	R8	—	—	—
	Control	V2	V6	R3-R4	R5	R6	R8	—	—	—
	References	V2	V6-V7	R3-R4	R5	R6	R8	—	—	—

[1] Site codes are as follows: PA = Berks County, PA; WI = Walworth County, WI.
[2] Obs. = Observation number; dates in month/day/year format.
- Indicates information not available.

Table G-5. Abiotic Stressor Evaluation Using Observational Severity Scale for MON 87705 and the Conventional Soybean Control

Abiotic stressor	Number of observations across the sites	Number of observations where no differences were detected between MON 87705 and the control
Total	**167**	**167**
Chloride toxicity	4	4
Cold	2	2
Drought	37	37
Flood	3	3
Frost	4	4
Hail	30	30
Heat stress	27	27
Moisture stress	4	4
Nutrient deficiency	12	12
Soil compaction	8	8
Wet soil	4	4
Wind	32	32

Note: The experimental design was a randomized complete block with three replications. Observational data were collected at four crop development stages: Observation 1 = V2-V4, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8. No differences were observed between MON 87705 and the control during any observation. Data were not subjected to statistical analysis.

Table G-6. Disease Damage Evaluations Using an Observational Severity Scale for MON 87705 and the Conventional Soybean Control

Disease	Number of observations across the sites	Number of observations where no differences were detected between MON 87705 and the control
Total	**206**	**203**
Alternaria leaf spot	14	14
Anthracnose	2	2
Asian rust	3	3
Bacterial blight	22	19*
Brown stem rot	5	5
Brown stem rust	1	1
Charcoal rot	2	2
Downy mildew	14	14
Cercospora	6	6
Frogeye leaf spot	31	31
Phytophthora[1]	10	10
Powdery mildew	11	11
Pythium	7	7
Rhizoctonia[2]	8	8
Sclerotinia	2	2
Seedling blight	1	1
Septoria (brown spot)	25	25
Soybean cyst nematode	1	1
Sudden death	12	12
Soybean mosaic virus	9	9
White mold	9	9
Soybean rust	11	11

*Indicates a difference observed between test and the control for bacterial blight at MO2 site (slight vs. none; Observation 1, Observation 2, and 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: Observation 1 = V2-V4, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8.
[1] includes *Phytophthora* root rot.
[2] includes *Rhizoctonia* root rot.

Table G-7. Arthropod Damage Evaluated Using an Observational Severity Scale for MON 87705 and the Conventional Soybean Control

Arthropod	Number of observations across the sites	Number of observations where no differences were detected between MON 87705 and the control
Total	**206**	**204**
Aphids[1]	28	27*
Armyworms	7	7
Bean leaf beetles	46	46
Flea beetles	1	1
Grasshoppers	26	26
Green cloverworms	20	20
Japanese beetles	28	28
Leafhoppers[2]	14	13*
Leafrollers	3	3
Mexican bean beetles	3	3
Soybean loopers	5	5
Spider mites	4	4
Stink bugs	11	11
Thistle caterpillars	2	2
Thrips	1	1
Whiteflies	2	2
Wireworms	1	1
Yellow wooly bear	4	4

*Indicates a difference observed between MON 87705 and the control for aphids (none vs. slight; Observation 2) and leafhoppers (none vs. slight; Observation 2) at the WI site. 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: Observation 1 = V2-V4, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8. Data were not subjected to statistical analysis.

[1] Includes soybean aphids.

[2] Includes potato leafhoppers.

Table G-8. Abundance of Pest Arthropods in Beat Sheet Samples Collected from MON 87705, the Conventional Soybean Control, and the Reference Soybean Varieties

		Abundance of Pest Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
Arthropod	Site[1]	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range
Aphid	IL2	31.7 (31.7)	18.3 (14.5)	5.0 – 25.3	—	—	—	—	—	—
	MO2	—	—	—	145.0 (123.2)	35.3 (32.4)	16.7 – 199.0	—	—	—
Bean leaf beetle	IA1	0.0 (0.0)	0.3 (0.3)	0.0 – 1.0	1.3 (0.3)	0.7 (0.3)	0.0 – 1.3	0.0* (0.0)	1.7 (1.2)	0.0 – 2.0
	IL2	12.0 (3.5)	12.0 (2.6)	8.7 – 12.0	11.0 (1.0)	10.3 (2.9)	7.3 – 16.0	17.0 (0.6)	22.7 (10.9)	8.7 – 21.3
	IN1	3.7 (1.5)	2.0 (1.0)	1.7 – 3.0	1.0 (0.6)	0.0 (0.0)	0.3 – 1.0	62.0 (18.0)	71.3 (17.9)	37.0 – 135.3
	MO2	3.7(1.2)	2.3(0.7)	2.3 – 5.0	0.7(0.3)	0.7(0.7)	0.0 – 1.3	4.7 (2.2)	3.3 (1.7)	2.0 – 5.0
Corn flea beetle	IN1	—	—	—	—	—	—	0.3 (0.3)	0.0 (0.0)	0.3 – 1.7
Green cloverworm	IA1	1.0 (0.0)	1.7 (0.3)	0.7 – 1.3	1.7 (1.2)	0.3 (0.3)	0.0 – 1.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.0
	IL2	0.0† (0.0)	0.0 (0.0)	0.0 – 0.0	2.0 (0.6)	1.3 (0.9)	0.0 – 2.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
	IN1	0.7 (0.3)	0.0 (0.0)	0.0 – 0.3	5.7 (2.0)	8.3 (2.6)	1.7 – 7.3	0.0 (0.0)	1.3 (1.3)	0.0 – 1.0
	MO2	7.7 (1.8)	4.3 (1.7)	6.7 – 8.7	3.3 (2.4)	1.7 (1.7)	1.0 – 5.3	1.3 (0.9)	2.7 (1.8)	0.3 – 1.7
Japanese beetle	IN1	0.3 (0.3)	1.3 (0.7)	0.0 – 2.0	2.3 (1.9)	2.0 (0.6)	0.7 – 4.7	—	—	—
Potato leafhopper	IA1	1.3 (0.3)	1.0 (1.0)	0.0 – 2.7	—	—	—	—	—	—
	IL2	16.0 (3.5)	30.0 (3.5)	25.7 – 117.3	—	—	—	—	—	—
	IN1	2.3 (1.5)	5.7 (2.8)	0.0 – 5.7	2.3 (2.3)	2.3 (0.3)	3.0 – 8.3	—	—	—

* Indicates a statistically significant difference between MON 87705 and the control ($p < 0.05$).
Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at three crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection 3 = R6-R8.
[1] Site codes are as follows: IA1 = Jefferson County, IA; IL2 = Stark County, IL; IN1 = Boone County, IN; MO2 = Lincoln County, MO.
[2] MON 87705 and control values represent mean number of arthropods collected across three replications. S.E. = standard error. Means based on n = 3.
† No statistical comparisons were made due to lack of variability in the data.
- Indicates the arthropod was not evaluated.

Table G-8 (continued). Abundance of Pest Arthropods in Beat Sheet Samples Collected from MON 87705, the Conventional Soybean Control, and the Reference Soybean Varieties

Arthropod	Site[1]	Abundance of Pest Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
		MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range
Stink bug	IA1	0.3(0.3)	0.0 (0.0)	0.0 – 1.0	0.0 (0.0)	0.7 (0.7)	0.0 – 1.0	0.3 (0.3)	0.0 (0.0)	0.0 – 1.3
	IL2	0.3 (0.3)	0.0 (0.0)	0.0 – 0.7	2.0 (1.0)	0.7 (0.7)	1.3 – 3.7	2.7 (0.9)	1.3 (0.9)	2.0 – 3.3
	IN1	0.0 (0.0)	0.0 (0.0)	0.0 – 0.7	1.0 (0.0)	0.3 (0.3)	0.0 – 0.3	4.3 (1.9)	6.0 (1.2)	1.0 – 3.7
	MO2	0.3 (0.3)	0.3 (0.3)	0.0 – 1.0	0.3 (0.3)	1.0 (0.6)	0.0 – 1.7	1.0 (1.0)	3.0 (1.0)	1.0 – 3.0
Thrips	IA1	23.0 (3.1)	11.7 (6.6)	5.0 – 29.3	—	—	—	—	—	—
	MO2	42.0 (21.0)	66.0 (54.9)	15.3 –34.0	—	—	—	—	—	—

Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at three crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection 3 = R6-R8.

[1] Site codes are as follows: IA1 = Jefferson County, IA; IL2 = Stark County, IL; IN1 = Boone County, IN; MO2 = Lincoln County, MO.

[2] MON 87705 and control values represent mean number of arthropods collected across three replications. S.E. = standard error. Means based on n = 3.

- Indicates the arthropod was not evaluated.

Table G-9. Abundance of Beneficial Arthropods in Beat Sheet Samples Collected from MON 87705, the Conventional Soybean Control, and the Reference Soybean Varieties

Arthropod	Site[1]	Abundance of Beneficial Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
		MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range
Big-eyed bug	MO2	1.0 (0.6)	1.0 (0.6)	0.3 – 3.0	5.3 (2.3)	7.7 (3.2)	8.0 – 9.0	7.3 (6.4)	8.0 (2.5)	10.7 – 19.7
Carabid beetle	IL2	—	—	—	—	—	—	0.3 (0.3)	0.3 (0.3)	0.3 – 1.0
	IN1	—	—	—	—	—	—	0.7 (0.7)	0.3 (0.3)	0.0 – 9.3
Lacewing	IA1	—	—	—	0.3 (0.3)	0.3 (0.3)	0.0 – 0.7	—	—	—
	MO2	—	—	—	1.0 (0.6)	1.0 (1.0)	0.7 – 1.7	0.0 (0.0)	0.3 (0.3)	0.0 – 1.3
Ladybird beetle	IA1	—	—	—	0.7 (0.3)	0.3 (0.3)	0.0 – 1.0	—	—	—
	IL2	—	—	—	0.0 (0.0)	0.7 (0.7)	0.3 – 4.3	0.7 (0.7)	0.7 (0.7)	0.0 – 2.0
	MO2	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	1.7 (1.7)	0.7 (0.7)	0.3 – 3.0	—	—	—
Micro-parasitic hymenoptera	IL2	—	—	—	1.3 (0.7)	0.7 (0.3)	0.7 – 2.3	—	—	—
Nabis	IA1	0.3 (0.3)	2.3 (1.9)	0.7 – 2.0	2.0 (1.2)	2.0 (0.6)	1.0 – 2.7	0.3 (0.3)	0.3 (0.3)	0.0 – 0.3
	IL2	0.0 (0.0)	0.7 (0.3)	0.0 – 2.3	0.7 (0.3)	1.3 (0.7)	0.3 – 2.3	0.7 (0.7)	1.0 (1.0)	0.0 – 1.0
	IN1	0.3 (0.3)	0.3 (0.3)	0.0 – 1.3	1.0 (0.0)	1.3 (0.7)	0.3 – 2.0	5.0 (2.0)	3.7 (1.9)	2.3 – 16.0
	MO2	0.3 (0.3)	1.0 (0.6)	0.3 – 2.3	1.3 (0.9)	1.7 (0.3)	0.0 – 1.7	0.3 (0.3)	0.3 (0.3)	0.3 – 1.3
Orius	IA1	0.3 (0.3)	0.0 (0.0)	0.0 – 0.3	0.3 (0.3)	0.3 (0.3)	0.0 – 0.7	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
	IL2	25.7 (15.7)	23.3 (4.8)	21.3 – 35.0	32.0 (4.0)	32.0 (7.5)	25.3 – 65.0	4.0 (1.0)	4.7 (2.0)	5.0 – 12.0
	IN1	3.0 (0.6)	5.7 (1.7)	1.0 – 3.3	14.3 (5.2)	17.3 (0.9)	8.7 – 23.0	7.3 (3.3)	1.3 (0.9)	1.0 – 31.3
	MO2	1.0 (1.0)	2.0 (2.0)	0.0 – 1.3	3.3 (1.8)	1.3 (0.7)	1.0 – 4.0	0.0 (0.0)	0.3 (0.3)	0.0 – 0.3

Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at three crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection 3 = R6-R8.

[1] Site codes are as follows: IA1 = Jefferson County, IA; IL2 = Stark County, IL; IN1 = Boone County, IN; MO2 = Lincoln County, MO.

[2] MON 87705 and control values represent mean number of arthropods collected across three replications. S.E. = standard error. Means based on n = 3.

- Indicates arthropod not evaluated.

Table G-9 (continued). Abundance of Beneficial Arthropods in Beat Sheet Samples Collected from MON 87705, the Conventional Soybean Control, and the Reference Soybean Varieties

Arthropod	Sites[1]	Abundance of Beneficial Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
		MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range	MON 87705 Mean (S.E.)	Control Mean (S.E.)	Reference Range
Spider	IA1	0.0 (0.0)	0.0 (0.0)	0.0 – 0.7	0.3 (0.3)	1.0 (1.0)	0.0 – 0.3	0.0 (0.0)	0.0 (0.0)	0.0 – 1.0
	IL2	1.7 (0.9)	1.3 (0.7)	0.7 – 3.3	1.0 (1.0)	1.7 (0.3)	1.0 – 2.3	0.0 (0.0)	1.7 (0.9)	1.3 – 2.7
	IN1	0.3 (0.3)	1.0 (0.6)	0.0 – 2.3	0.3 (0.3)	0.3 (0.3)	0.3 – 1.0	1.7 (0.9)	2.7 (0.3)	0.3 – 3.7
	MO2	0.3 (0.3)	0.7 (0.3)	0.0 – 2.0	1.3 (0.3)	2.0 (0.6)	0.0 – 2.3	2.0 (1.2)	3.3 (0.7)	0.3 – 3.7

Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at three crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection 3 = R6-R8.

[1] Site codes are as follows: IA1 = Jefferson County, IA; IL2 = Stark County, IL; IN1 = Boone County, IN; MO2 = Lincoln County, MO.

[2] MON 87705 and control values represent mean number of arthropods collected across three replications. S.E. = standard error. Means based on n = 3.

- Indicates arthropod not evaluated.

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

H.1 Plant Production

MON 87705, a conventional soybean control (A3525), and five commercially available reference soybean varieties were grown in St. Louis County, MO, in a randomized complete block design with three replications. Each plot consisted of four rows approximately 20 ft in length.

H.2 Flower Collection

When soybean plants were at flowering stage, whole flowers were collected from five non-systematically selected plants from the first and fourth row of each plot. 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 labelled 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 eight hours until the pollen was prepared and stained.

H.3 Pollen Sample Preparation

Pollen samples were prepared in a laboratory. Clean microscope slides were labelled 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 and a dissecting needle 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.

H.4 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 [Olympus Optical Co., Ltd.] and imaging software Image-Pro Plus v4.5.1.27 [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. Nonviable 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 nonviable pollen grains was counted from a minimum of 75 pollen grains from a random field of view under the

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

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 87705, the conventional soybean control, and commercial reference soybean varieties that were also used for pollen diameter measurements.

H.5 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% ($p<0.05$). MON 87705 was compared to the conventional soybean control for percent viable pollen and pollen diameter. No statistical comparisons were made between MON 87705 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.

Appendix H References

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

SAS. 2002-2003. SAS Software Release 9.1 (TS1M0), SAS Institute, Inc., Cary, North Carolina.

Appendix I. Materials and Methods for Symbiont Evaluation

I.1 Materials

The starting seed of MON 87705 was produced in Puerto Rico in 2006-2007 under Protocol LS272 by Monsanto Trait Development. The control substance starting seed was produced in Illinois in 2007 under Monsanto Production Plan 07-01-83-18. Reference substance starting seed was obtained from commercial sources or was produced in Puerto Rico in 2007 under Seed Increase 07-SI-71-01. Nodules, root tissue, and shoot tissue collected from MON 87705, the conventional soybean control, and reference soybean varieties were evaluated in the study.

Table I-1 Starting seed of MON 87705, Control, and Commercial Soybean Reference Varieties Used in the Symbiont Evaluation

Materials	Material Type	Material Source[1]	Phenotype
MON 87705	Test	Monsanto TD	Improved fatty acid profile
A3525	Control	07-01-83-18	Conventional
A2553	Reference	Commercial	Conventional
Stine 3300-0	Reference	07-SI-71-01	Conventional
Stewart SB3454	Reference	Commercial	Conventional
Garst 3585N	Reference	Commercial	Conventional
Hartz H5218	Reference	07-SI-71-01	Conventional
A5560	Reference	07-SI-71-01	Conventional

[1] Starting seed were obtained from commercial sources, Monsanto Trait Development (TD), Monsanto Field Production 07-01-83-18, or Monsanto Seed Increase 07-SI-71-01.

The presence or absence of MON 87705 in the test and control starting seed was verified by event-specific polymerase chain reaction (PCR) analyses. Results of PCR analyses were as expected.

I.2 Greenhouse Phase and Experimental Design

MON 87705, the conventional soybean control, and reference soybean varieties starting seed were planted in 6-inch pots containing nitrogen-deficient potting medium (Sunshine Mix #2 Basic/LB2, Sun Gro Horticulture, Inc., Garland, TX) comprised of primarily peat, vermiculite, and perlite. Plants from MON 87705, the conventional soybean control, and reference soybean varieties starting seed were grown in a greenhouse where actual temperatures ranged from approximately 21 ^{o}C to approximately 42 ^{o}C. Eight replicate pots were planted with three seeds per pot for each of MON 87705, the conventional soybean control, and reference soybean varieties. At planting, each seed was inoculated

with approximately 1 x 10^7 cells of *Bradyrhizobium japonicum* (VAULT NP, Becker Underwood, Ames, IA) in phosphate-buffered saline. Pots were arranged after thinning 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 September 9 and 10, 2008, and MON 87705 and the conventional soybean control starting seed were planted on September 11, 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.

I.3 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 labelled 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 72 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 72 hours at approximately 65 °C for dry weight determination. The shoot tissue was ground after drying with a Harbil 5G high-speed paint shaker prior to total nitrogen analysis. Shoot total nitrogen was determined by combustion using a nitrogen analyzer (Rapid N Cube, Elementar Americas, Inc.).

I.4 Statistical Analysis

The data consisted of six measurement endpoints taken at the six week sampling period: nodule number, nodule dwt (g), shoot dwt (g), root dwt (g), and shoot total nitrogen (% and g/plant). Data obtained from MON 87705, the control (A3525), and the 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 9.2, SAS Institute, Inc. 2002-2008) with the level of statistical significance predetermined to be 5% ($p<0.05$).

Appendix J. Materials and Methods for Analysis of *FAD2-1A* and *FATB1-A* RNA levels in MON 87705 immature seeds

J.1 Test Substance

The test substance was MON 87705. As listed below in the table, four replicates of immature seed from a production plan, generated from seed lot GLP-0702-18254-S were used in this study.

Sample ID	Seed Lot #	Tissue Type	Replicate #
REG07189-0015	GLP-0702-18254-S	Immature seed	1
REG07189-0016	GLP-0702-18254-S	Immature seed	2
REG07189-0017	GLP-0702-18254-S	Immature seed	3
REG07189-0018	GLP-0702-18254-S	Immature seed	4

J.2 Control Substance

The control substance was conventional soybean variety A3525, the same genetic background as the test substance. As listed below in the table, four replicates of immature seed from a production plan, generated from seed lot GLP-0702-18252-S were used in this study.

Sample ID	Seed Lot #	Tissue Type	Replicate #
REG07189-0001	GLP-0702-18252-S	Immature seed	1
REG07189-0002	GLP-0702-18252-S	Immature seed	2
REG07189-0003	GLP-0702-18252-S	Immature seed	3
REG07189-0004	GLP-0702-18252-S	Immature seed	4

J.3 Reference Substance

Probe templates were used as positive hybridization controls in northern blot analyses. Prior to probe generation, DNA segments from the *FAD2-1A* (Monsanto Sequence Database) and *FATB1-A* (Monsanto Sequence Database) genes were generated from conventional soybean genomic DNA using a forward primer specific to the coding region and a reverse primer specific to the 3'UTR region of each gene. The resulting PCR segments of the *FAD2-1A* and *FATB1-A* genes were cloned into pGEM-T Easy vectors (pGEM-T Easy Vector System, Invitrogen, Carlsbad, CA) according to the manufacture's manual with minor modifications documented in the raw data. The modifications were approved by the study director and documented in the raw data. The sequence of the plasmid DNA was confirmed by sequencing and comparison to the expected sequences using pair wise alignments. The plasmid DNAs then were used as templates for PCR reactions to amplify the FAD2-1A and FATB1-A probe templates. The actin probe template was generated by PCR from conventional soybean genomic DNA using a forward primer specific to the coding region and a reverse primer specific to the 3'UTR region of the soybean *actin* gene (Soy57, GI: 1498333). All PCR reactions and purification of DNA segments from agarose gels were performed according to in house SOPs. RiboRuler™ RNA ladders (high range) from

Fermentas Co (Glen Burnie, MD) were used for size estimations on northern blots and formaldehyde/agarose gels. The unique identities of the probes, as well as molecular weight markers are documented in the raw data.

J.4 Characterization of Test, Control and Reference Substances

The identity of the test and control substances was determined by event-specific PCR prior to planting the seed in the green house. The study director reviewed the chain of custody (COC) documentation and certificate of analysis (COA) to confirm the identity of the test and control substances prior to use of the materials in the study and a copy of the COAs was archived with the study data. Immature seed was harvested from the plants and identity confirmation was performed on each sample by event-specific PCR prior to use in the study. The study director reviewed the data prior to use of the materials in the study and the raw data was archived with the study. Mature seed was harvested from the plants and tested by an event-specific PCR. These results were reviewed by the study director prior to the start of the study and a copy of the Verification of Identity (VOI) was archived with the study.

Prior to the start of the study, the FAD2-1A, FATB1-A, and actin probe templates were sequenced by the Monsanto Genomics Sequencing Center and the sequences were aligned with the expected sequences to confirm the identities of the probes. The raw data are archived with the study.

Extracted RNA and RNA markers were stored in a -80° C freezer. DNA probes were stored in a -20° C freezer. The test, control, and reference substances were deemed stable during storage because there were no signs of degradation on formaldehyde/agarose gels and probing with endogenous genes yielded interpretable signals on northern blots.

J.5 Total RNA extraction

Total RNA from test and control substances was extracted according to an in-house SOP. All extracted RNA was stored in -80° C freezers.

J.6 PolyA+ RNA Isolation

PolyA+ RNA[10] from the test and control substances was extracted from total RNA using the Poly(A) Purist MAG Kit (Ambion Co., Austin, TX) according to the manufacturer's protocol with minor modifications approved by the study director prior to performing the analysis. The procedure was documented in the raw data. The resulting polyA+ RNA was stored in a -80 °C freezer.

J.7 Formaldehyde/Agarose Gel Electrophoresis

PolyA+ RNA samples from test and control substances for *FAD2-1A* northern blot analysis were extracted from approximately 20-50 µg of total RNA, and resolved in 0.8% formaldehyde/agarose gels (0.8% with regard to agarose). PolyA+ RNA samples from test and control substances for *FATB1-A* northern blot analyses were extracted from approximately 100 µg total RNA, and resolved on 1.0% formaldehyde/agarose gels (1.0% with regard to agarose).

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[10]Poly A enriched RNA

J.8 Northern Blot Analyses

Northern blot analyses were performed by transferring polyA+ RNA from formaldehyde/ agarose gels onto nylon membranes and probing with radiolabeled DNA. FAD2-1A and FATB1-A hybridization signals were stripped from the blots and the stripped blots were hybridized to a ^{32}P-labeled actin probe in order to show the relative amount of RNA loaded in each lane. Probes were prepared by random prime labeling. Each probe was added at approximately 1-2 × 10^6 cpm per ml of the hybridization solution.

DNA probe templates were run on the formaldehyde/agarose gels to serve as positive controls for hybridization. On northern blots the probe templates migrated slightly differently than predicted by the RNA ladder. The difference in migration is probably due to the inaccuracy of comparing a DNA probe to an RNA ladder. The following table lists the DNA probes, the positive hybridization controls, and the hybridization and wash temperatures for each northern blot analysis.

Analysis	Positive hybridization controls	Probe	Hybridization and Wash Temperature
Expression of *FAD2-1A*	FAD2-1A probe template	FAD2-1A	65 ºC
Expression of *FATB1-A*	FATB1-A probe template	FATB1-A	60 ºC
Expression of *actin*	actin probe template	actin	55 ºC

Appendix K. Manufacturing Process for Soybean

MON 87705 may be processed using conventional industry standard processing methods, which include extrusion methods (Erickson et al., 1980). Following cracking and aspiration of the soybean to separate the hulls, the hulls are screened to recover the fines generated during cracking, and the cracked soybean meats are conditioned and flaked to rupture oil cells and prep are a thin flake with a large surface area for solvent extraction. The soybean flakes then undergo solvent extraction with iso-hexane/hexane to yield crude soybean oil and soy meal. The crude oil is processed through a series of steps known as refining. In the first step, phospholipids are removed through a process known as degumming. This involves mixing with water to form gums. followed by centrifugation. The fatty acids in the degummed oil are neutralized through the addition of a caustic solution (sodium hydroxide) to form soaps soluble in water in a process known as alkali-refining. This process is preceded by a pretreatment step with phosphoric acid. The resulting soap solution is removed by centrifugation. Water washing and centrifugation further removes soaps to levels compatible with bleaching. Alternatively, oil can be further treated with trisyl to remove any residual soap content and then filtered. In the next step, the oil is "bleached" by mixing with a citric acid solution, followed by treatment with adsorbent clay to remove the peroxides, phosphatides, color bodies and traces of soap. Under vacuum to inhibit oxidation, the pigments are adsorbed and removed by filtration. In the final refining step, odoriferous components, flavor components and additional free fatty acids are removed by steam distillation to produce refined, bleached and deodorized (RBD) oil. This process of deodorization is carried out at high temperatures (250°C) and under vacuum. Anti-oxidants are utilized to inhibit oxidation of the oil. A pictorial representation of the manufacturing process described above is shown below in Figure K-1.

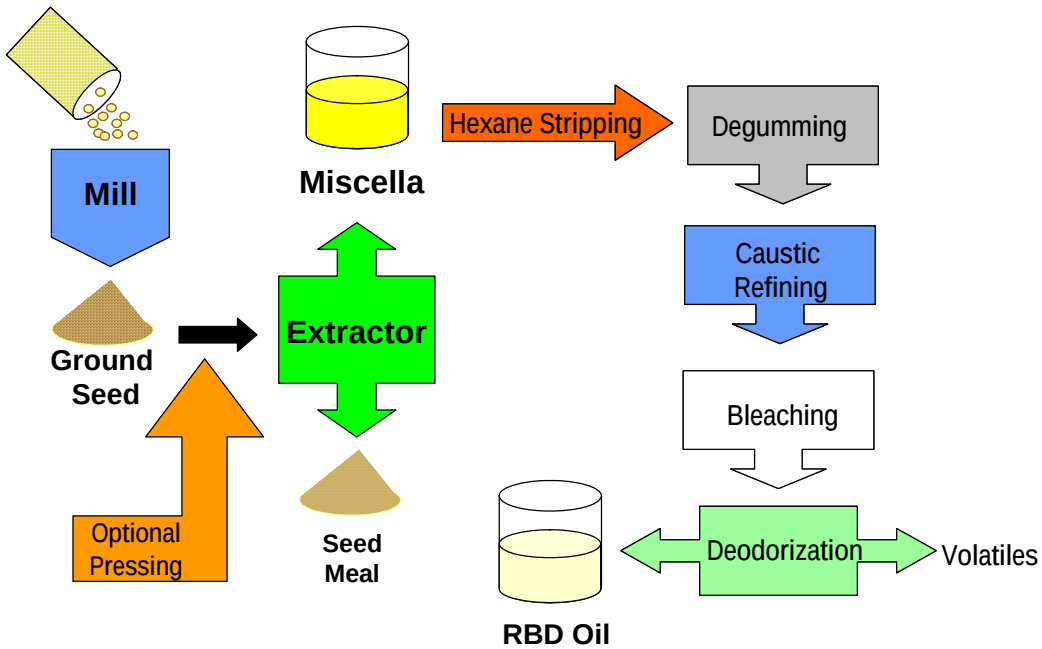

Figure K-1. Schematic of Manufacturing Process for Soybean Oil.

Appendix K References

Erickson, D.R., E.H. Pryde, O.L. Brekke, T.L. Mounts and R.A. Falb, eds. 1980. Handbook of Soy Oil Processing and Utilization. American Soybean Association and American Oil Chemist's Society, St. Louis, Missouri and Champaign, Illinois. Pp. 49-130.

Appendix L. Glyphosate Resistance

L.1 Introduction

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. Stewardship of the glyphosate molecule to preserve its usefulness for growers is an important aspect of Monsanto's stewardship commitments. While herbicide resistance may eventually occur when any herbicide is widely used, resistance can be postponed, contained and managed through research, education and good management practices. These are key elements of Monsanto's approach to glyphosate stewardship.

The development of plant populations resistant to glyphosate can adversely impact the utility and life cycle of glyphosate products if it is not managed properly. As leaders in the development and stewardship of glyphosate products for over 30 years, Monsanto makes a significant investment in research to understand and develop the proper use and stewardship of glyphosate. Monsanto also invests in grower/retailer education and training programs to provide information on best practices to manage glyphosate resistance. This research includes an evaluation of factors that can contribute to the development of weed resistance and develop ways to mitigate the risk. The risk of weeds developing resistance and the potential impact of resistance on grower weed management practices vary greatly across different herbicide modes of action and they depend on a combination of factors. Therefore, there is no single set of best management practices to mitigate resistance for all herbicides, nor is there a single set of best management practices to mitigate resistance to a single herbicide for all weeds in all cropping systems. However, this document provides an overview of Monsanto's approach to the development of best management practices to mitigate glyphosate resistance.

L.2 The Herbicide Glyphosate

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #: 1071-83-6), the active ingredient in the Roundup family of nonselective, foliar-applied, postemergent agricultural herbicides, is among the world's most widely used herbicidal active ingredients. Glyphosate is highly effective against the majority of economically significant annual and perennial grasses and broadleaf weeds. Currently glyphosate is labeled for control of more than 300 weed species. Glyphosate kills plant cells by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Franz et al., 1997). Glyphosate is the only known herbicide with this mode of action (Franz et al., 1997). The relevant aromatic amino acid pathway is not present in mammalian metabolic systems (Cole, 1985). A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development, and does not bioaccumulate in mammals (Williams et al., 2000). An ecotoxicological risk assessment concluded that the use of glyphosate does not pose an unreasonable risk of adverse effects to nontarget species, such as birds and fish, when used according to label directions (Giesy et al., 2000). Glyphosate has favorable environmental characteristics, including a low potential to move through the soil to reach ground water and is degraded over time by soil microbes. Because it binds tightly to soil, glyphosate's

bioavailability is reduced immediately after application, which is why glyphosate has no residual soil activity.

L.3 Herbicide Use and Herbicide-resistant Weeds

The vast majority of U.S. soybean growers depend, at least in part, on herbicides to control weeds. According to a report by USDA-ERS, herbicides were used on more than 97 percent of the total U.S. soybean acreage in 1997 (Fernandez-Cornejo and McBride, 2002).

Control of weeds in a crop is essential because weeds compete with the crop for the same limited resources in the field, including sunlight, water and nutrients (Ross and Lembi, 1985; Wilcut et al., 2003). Lack of effective weed control in soybean fields can result in yield losses of 50-90%. Because failure to control weeds within the crop can result in decreased yields and reduced crop quality, an intensive program for weed control is essential to ensure profitability (Dalley et al., 2001). When failure to control weeds in a crop increases weed populations, additional herbicide applications or cultural practices are then required to reduce the weed population.

With any herbicide use, however, comes the potential for the selection of weeds resistant to that herbicide. Within a weed species individuals may possess an inherent ability to withstand the effects of a particular herbicide. Repeated use of that herbicide will expose the weed population to a "selection pressure," which may lead to an increase in the number of surviving resistant individuals in the population (HRAC, 2009). In other words, plants susceptible to the applied herbicide will die, while those few having some type of natural resistance may survive and reproduce.

Weed resistance is generally defined as the naturally occurring heritable ability of some weed biotypes within a given weed population to survive a herbicide treatment that should, under normal use conditions, effectively control that weed population. Thus, a resistant weed must demonstrate two criteria as defined by the Weed Science Society of America website at www.wssa.net: (1) the ability to survive application rates of a herbicide product that once were effective in controlling it, and (2) resistance is heritable. Procedures to confirm resistance generally require both field and greenhouse analysis. Obtaining a correlation between field and greenhouse studies has been particularly important for the accurate detection of glyphosate resistance, for which the levels of resistance observed have been as low as 2X the susceptible biotypes (Lee and Ngim, 2000; Lorraine-Colwill et al., 1999).

Herbicide-resistant weeds are neither a new phenomena nor is resistance unique to glyphosate. In 1952, the first U.S. herbicide-resistant weed, a spreading dayflower species resistant to the synthetic auxin 2,4-D, was identified in Hawaii (Heap, 2009). There are currently 72 herbicide-resistant weed species in the U.S. For example there are 38 weed species resistant to ALS herbicides, 15 species resistant to ACCase inhibitors, 23 weed species resistant to triazine herbicides, and nine (9) weeds species resistant to glyphosate herbicides (Heap, 2009). Growers have been managing these herbicide-resistant weeds for decades with the use of alternative herbicides and/or cultural methods such as tillage or crop rotation.

The occurrence of a herbicide-resistant weed biotype generally does not end the useful lifespan or preclude the effective use of the herbicide in question as part of an overall weed management system. This is particularly true for glyphosate, which remains a very useful tool despite its lack of effectiveness on specific resistant weed biotypes due to its ability to control a weed at different growth stages and the wide spectrum of weeds it effectively controls. Even for other herbicides that do not have the spectrum of control or the wide window of application, growers have continued to have a need to use these products even with the presence of resistant populations. For example, in 1995, imazethapyr and imazaquin, both in the imidazolinone herbicide family, were used on almost 60 percent of the soybean acres in the U.S., even though, at that time, nine weed species that were resistant to those herbicides were present in soybean producing areas (Heap, 2009).

It is important to distinguish herbicide resistance, explained above, from herbicide tolerance. In contrast to species for which resistant biotypes have occurred, herbicide tolerant weed species has the inherent ability to survive applications of a particular herbicide at recommended rates. In other words, the species does not develop tolerance through selection but is innately tolerant to that herbicide. It may also be the case that a certain weed species, while neither resistant nor tolerant, is difficult to control with a particular herbicide, requiring more careful herbicide use and weed management. Section E herein discusses resistant, tolerant, and hard-to-control species in the context of glyphosate.

L.4 Characteristics of Herbicides and Herbicide Use Influencing Resistance

While the incidence of weed resistance is often associated with repeated applications of a herbicide product, the actual onset of resistance within a population depends very much on the specific herbicide chemistry in question, as well as the inherent presence of gene(s) that confer the ability of a plant to be resistant to a particular chemical within a specific weed species and even a specific population of that species (Sammons et al., 2007). Some herbicide products are much more prone to develop herbicide resistance than others (Heap, 2009). Glyphosate has been used extensively for over three decades with relatively few cases of resistance development, particularly when compared to many other herbicides, considering the substantial acreage glyphosate-treated worldwide, and the total number of weeds that glyphosate can control. The graph in Figure 1 illustrates the instances of weed resistance to various herbicide families. The different slopes observed are largely due to the factors described above, which relate to chemistry and function, in addition to levels of exposure in the field. The summary below describes herbicide-specific factors determined to be important in the process of selecting for individuals that are inherently resistant to a herbicide.

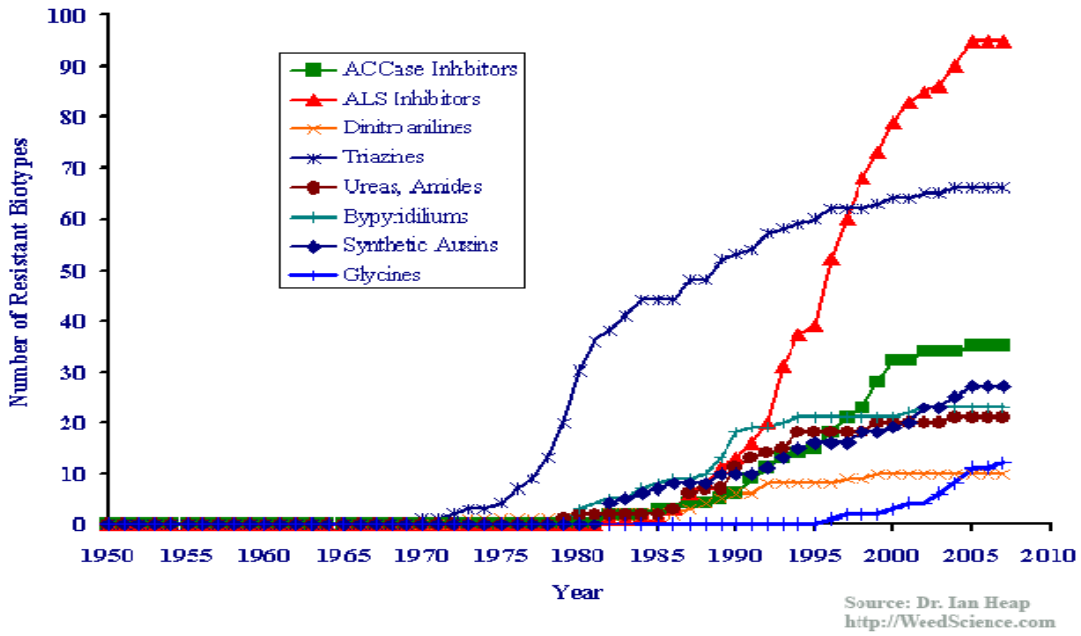

Figure L-1. Weed Resistance to Various Herbicide Chemical Families

L.5 Mechanisms of Resistance

The application of a herbicide to a weed does not, itself, cause a mutation in later generations of the plant. Rather, over time, those few biotypes that are not susceptible to the herbicide may become dominant within a population with the repeated use of that herbicide. To date, the three known avenues by which a weed species is resistant to an herbicide have been identified as target site alteration (target site), enhanced metabolism of the herbicides (metabolism), and reduced absorption and/or translocation of the herbicide such that the herbicide does not get to the site of action within the plant cell (exclusion) (Sammons et al., 2007).

Herbicide resistance via target site is the most common resistance mechanism among the various herbicide classes. It has been found that a target site mechanism is the most common mechanism for ALS inhibitors, ACCase inhibitors, and triazines, but is less common for glyphosate. A target site alteration is one where there is/are amino acid substitution(s) in the protein that is the target of a herbicide such that the alteration prevents the binding of the herbicide to the protein and thus the activity of the protein is not altered and the plant grows normally. For ALS inhibitors, the level of resistance conferred by a target site mechanism has been found to be as high as 3,400 X (Ferguson et al., 2001). (Note: X is the labeled or recommended rate for a herbicide on a particular weed species.) For glyphosate, species found to exhibit a target site mechanism often show low levels of resistance (2-3X) due to the fact that glyphosate is a true transition state inhibitor (Schonbrunn et al., 2001; Sammons et al., 2007). In addition, multiple alterations of the same enzyme have been found for ALS and ACCase inhibitors (Tranal and Wright, 2002). This may explain the apparent high frequency of resistance and the short time in which resistance developed to herbicides in these two

classes of chemistries. Only one altered site in the targeted plant EPSPS enzyme has been found for glyphosate (Baerson et al., 2002).

Herbicide resistance as a result of exclusion mechanisms is the glyphosate resistant mechanism among the majority of the weed species studied to date. This resistance mechanism has also been found to be associated with 2,4 D and paraquat. Within this category, there are two types of translocation alterations that have been observed for glyphosate; (a) restricted movement of glyphosate from leaf cells into the meristematic cells of the plant and (b) restricted movement of glyphosate within a cell into the chloroplast due to accumulation within the vacuole. The level of glyphosate resistance conferred with this mechanism is higher (6-8X) than for species exhibiting target site mutations (2-3X).

The third type of herbicide resistance mechanism, metabolism, has not been found to be a resistance mechanism associated with glyphosate in any of the weed species studies thus far. However, legumes have been shown to degrade glyphosate and therefore this type of resistance mechanism may be active in some species (Reddy et al., 2008).

In some species the experimental evidence suggests that multiple mechanisms of glyphosate resistance may occurr within the same plant and multiple mechanisms are needed to protect the plant from the phytotoxic effects of glyphosate (Yu et al., 2007). This implies that multiple genes (polygenic resistance) are necessary and thus the selection of plants with multiple genes needed to confer resistance would be expected to occur at a low frequency.

In summary, the overall low occurrence of glyphosate resistance may be in part explained by: (1) the nature of the target site inhibition by glyphosate relative to other herbicides, (2) the lack of metabolism as a mechanism of selectivity for weed resistance, and (3) evidence of multiple mechanisms being necessary for resistance and thus resistance is polygenic and difficult to assemble and maintain.

L.6 Use of Recommended Glyphosate Rate

The interaction between herbicide application rate and resistance for postemergence herbicides, such as glyphosate, is dependent upon the nature of the plant gene(s) conferring resistance to the chemical. In general, herbicide rate has more effect on selecting for resistant individuals in a population if the resistant gene is semi-dominant or recessive as compared to the resistant gene being dominant. Likewise, herbicide rates would have more of an effect on the onset of resistance if commercially significant resistance required the additive effect of multiple genes (i.e. quantitative or polygenic resistance). Low rates would tend to allow certain biotypes to survive and mate with other biotypes of the same or an alternate resistant gene. The offspring of this mating may then be able to survive a full rate.

Less-than-recommended or suboptimal rates have been implicated or speculated as the causal factor in herbicide resistance for several different herbicides, including chlortoluron-resistant blackgrass, diclofop-resistant ryegrass and dicamba-resistant kochia (Beckie, 2006). Busi (2009) demonstrated that, in three generations of a ryegrass biotype sprayed at sublethal rates of diclofop-methyl or glyphosate, a high level of resistance evolved to diclofop-methyl and a moderate level to glyphosate. The conclusion of this work was that growers should avoid lowering the application rate of herbicides, especially where major cross-pollinating weed species, such as lolium, are present.

L.7 Weeds Resistant to Glyphosate

As with any other herbicide, the use of glyphosate may lead to the development of glyphosate-resistant weed species, and a list of glyphosate resistant weeds is provided below in Table L-1. However, the potential for the development of a glyphosate-resistant weed needs to be considered in the following context: (1) if a glyphosate-based weed control system were not available, other herbicide(s) with equal or greater potential for resistance would be used to control weeds and (2) other herbicides and cultural practices can be used to manage the glyphosate resistant species (Neve, 2008; Gustafson, 2008).

To date, biotypes of fifteen weed species resistant to glyphosate have been identified and confirmed worldwide. Nine species resistant to glyphosate have been confirmed in the U.S., two of which were identified outside of Roundup Ready cropping systems. The speed of spread and geographical distribution of the resistant species has varied. Some species with resistant biotypes, such as common ragweed (*Ambrosia artemisfolia*) have been found in a limited number of sites across the mid-west, whereas marestail (*Conyza cana*densis) has been found in many states in the northeast, mid-west and the south. The reproductive biology of the particular weed species involved appears to be a factor contributing to the spread of resistant biotypes. In the above examples, marestail produces a large number of wind-dispersed seeds, which contributes to rapid spread, while ragweed seeds do not have features that allow for such easy distribution by the wind (Weaver, 2001; Pennsylvania State University Cooperative Extension Service Weed Identification Bulletin #8, 1986).

Table L-1. U.S. Glyphosate Resistant Weeds through June 2009

Weeds identified outside of Roundup Ready Systems	Rigid ryegrass (*Lolium rigidum*)
	Hairy fleabane (*Conyza bonariensis*)
Weeds identified in Roundup Ready Systems	Horseweed (*Conyza canadensis*) Common ragweed (*Ambrosia artemisiifolia*) Giant ragweed (*Ambrosia trifida*) Palmer amaranth (*Amaranthus palmeri*) Common waterhemp (*Amaranthus rudis*) Italian ryegrass (*Lolium multiflorum*) Johnson grass (*Sorghum halepense*)

Some weed species, such as *Equisetum arvensi*s (field horseweed), are tolerant, as opposed to resistant, to herbicides. In addition, some species are more difficult to control with glyphosate than others (e.g. lambsquarters (*Chenopodium album*) and morninglory (*Ipomea sp.)* and require more care to make sure the correct amount of glyphosate is applied at the right growth stage. For these difficult-to-control weeds, environmental conditions can affect herbicide performance more than for weeds that are easier to control and therefore it is more critical that the correct rate be applied at the right growth stage when making applications to weeds in the difficult-to-control category. Weed control situations involving tolerant or difficult-to-control species are often confused with resistance.

L.8 Use of Glyphosate for In-crop Weed Management

Monsanto has developed plants through biotechnology to be tolerant to glyphosate. The development, approval and cultivation of these Roundup Ready crops have facilitated additional uses of glyphosate in crops where such uses were not previously possible given the non-selective nature of glyphosate. This development has provided growers with an additional weed management option and benefits relative to existing weed management options. The glyphosate-tolerant trait in Roundup Ready crops has no effect *per se* on the control of weeds. From a weed resistance standpoint, the use of glyphosate with glyphosate-tolerant soybean is no different than the use of a selective herbicide in a conventional soybean crop.

The most often cited benefits of glyphosate as an in-crop weed management option are simplicity, flexibility of application timing, weed spectrum, crop safety, and environmental safety (Dill, 2005). The ability to use glyphosate in-crop has allowed farmers to change their farming practices in some cases. For example, Roundup Ready cotton and Roundup Ready soybean have often been cited as a major reason for an increase in no-till practices (Dill et al., 2008).

Since Monsanto commercialized the first Roundup Ready soybean variety in 1996, growers have enthusiastically adopted the technology. Currently, biotechnology-derived herbicide tolerant soybean is planted on over 92% of U.S. soybean acreage (USDA-NASS, 2008). The Roundup Ready soybean system, (i.e., planting Roundup Ready soybean and applying glyphosate in-crop), has become the standard weed control program in U.S. soybean production. In addition, weed control in a Roundup Ready soybean system likely will involve not only glyphosate-based herbicides but also other herbicides and weed management practices to effectively manage weeds, thus increasing crop yield and reducing development of resistant weeds. State Universities/Cooperative Extension Services (CES) publish information on best weed management practices in Roundup Ready crops to address both of these objectives (see Table L-2). In addition Monsanto and other companies selling glyphosate products provide information on these same best management practices as detailed later in this Appendix.

L.9 Weed Resistance Management Strategies for Glyphosate

As part of Monsanto's stewardship of Roundup agricultural herbicides and Roundup Ready crop systems, the company has conducted investigations and worked extensively with

academics and other herbicide manufactures to understand the best practices to manage resistance. These investigations have demonstrated that one of the major factors contributing to the development of resistant weeds is poor weed control management practices. These include application of herbicides at rates below those indicated on the EPA-approved label for the weed species and sole reliance on a particular herbicide for weed control without the use of other herbicides or cultural control methods (i.e. pre-plant and in-crop tillage) (Beckie, 2006; Peterson et al., 2007).

The weed resistance management recommendations that will be made for the use of glyphosate in conjunction with varieties developed from MON 87705 will be consistent with the Herbicide Resistance Action Committee's guidelines for prevention and management of herbicide resistance (HRAC, 2009). These guidelines recommend an integrated approach to weed resistance management including crop management (i.e. row spacings, etc), cultural techniques and herbicides.

EPA is the U.S. federal regulatory agency that administers the federal law governing pesticide sale and use (FIFRA). EPA encourages pesticide manufacturers to provide growers with information regarding a herbicide's mode of action to aid growers in planning herbicide use practices and to foster the adoption of effective weed-resistance management practices as specified by EPA in PR Notice 2001-5. In that document EPA states that "this approach to resistance management is sound and would be highly beneficial to pesticide manufacturers and pesticide users" (EPA PR Notice 2001-5 at http://www.epa.gov/opppmsd1/PR_Notices/pr2001-5.pdf). EPA approves all pesticide label use instructions based on the agency's evaluation of supporting data supplied by the pesticide registrant or manufacturer. After EPA approves a pesticide label, it is a violation of federal law to use the pesticide for a use or in a manner not in accordance with the label directions.

Monsanto incorporates EPA's guidelines for pesticide resistance management labeling on its glyphosate-based agricultural herbicide labels, and will do so on the label for products to be applied over the top of varieties developed from MON 87705 (An example of current Roundup WeatherMAX product label is available at www.cdms.net/ldat/ld5UJ029.pdf). EPA-approved labels for Roundup branded herbicide weed-resistant management recommendations are designed to minimize the potential for the development of glyphosate-resistant weeds. By approving a label for a glyphosate-based agricultural herbicide, EPA has concluded that the product will not cause unreasonable adverse effects to the environment or human health when used in accordance with the label's directions.

The weed resistance management guidelines on the labels of Roundup agricultural herbicides include recommendations that are well-documented in the scientific literature as being appropriate and effective for weed control and to mitigate weed resistance. Significant research has been conducted to identify the appropriate application rate of glyphosate required to control a particular weed at various growth stages under various agronomic and environmental conditions. These rates are based on over 35 years of ongoing research at Monsanto to evaluate the efficacy of Roundup agricultural herbicides. Studies have included efficacy of weed control for a broad spectrum of weeds and under a wide range of conditions. Research has also involved the study of tolerance of the Roundup Ready crops to over-the-top applications of Roundup agricultural herbicides. Delaying herbicide application allows weeds to grow larger, requiring higher herbicide rates for control. At lower glyphosate application rates, larger weeds are controlled less consistently (Gubbiga et al., 2002). A key

element of effective weed control and weed resistance management, therefore, is using the correct rate of glyphosate at the right time for the weed species and the size of the weed (i.e., using a lethal dose which avoids the need for subsequent applications). This important strategy is well-supported by field research studies at several universities (Wilson et al., 2003; Jeschke et al., 2006; Stoltenberg, 2002; Wilson et al., 2006). Additionally, it is accepted in the weed science community that the use of multiple herbicide modes of action via tank mixtures, use of herbicides with different modes of action in a rotational crop, or using multiple herbicides in sequence within a crop will reduce the risk of developing weed resistance (Gessel and Stegal, 1990; Beckie, 2006). Tank-mixing involves mixing two or more herbicides in the spray tank immediately prior to application. Simultaneously using two herbicides with different modes of action significantly reduces the probability of weeds developing resistance to either or both chemistries. To provide growers with the tools needed to minimize resistant weed development, Monsanto will continue to investigate and recommend appropriate residual and postemergence herbicide products that have a different mode of action from glyphosate. As an example, the herbicide metolachlor (tradename PARRLAY™) is a residual herbicide that will help reduce flushes of annual grasses and pigweed which could slow the selection and potential spread of glyphosate-resistant weeds in Roundup Ready cotton and Roundup Ready Flex cotton systems.

Crop rotation and management of the fallow period and cover crops, has been found to be an important consideration in managing resistance. In general, crop rotation fosters use of alternate herbicide modes of action and, potentially, use of additional cultural practices to manage weeds over time (Beckie et al., 2004). Several authors have referred to this general concept as applying "diversity" across cropping/fallow seasons to manage weed resistance (Beckie, 2006; Powles, 2008). In general, conservation tillage practices (minimum-till and no-till) are viewed as creating environments where herbicide resistance is likely to develop. This is probably due to heavy selection pressure put on weeds by herbicides in these environments and the absence of tillage as a cultural practice to supplement herbicides. However, this is not always the case. Legere et al. (2000) found that an increase in ACCase inhibitors (aryloxyphenosy propionate and cyclohexanedinone chemical families) use in conservation tillage system did not result in an increased incidence of wild oat populations resistant to ACCase inhibitors. In addition, preplant and/or in-crop cultivation will actually promote the spread of some species such as Johnsongrass. Therefore, no-till would be a better cropping system to contain Johnsongrass populations that could contain a resistant gene (McWhorter, 1972).

The stewardship program for managing weed resistance in MON 87705 soybean is essentially the same program that is already in place for Roundup Ready soybean event 40-3-2 and for Roundup Ready 2 Yield soybean. The weed-resistance management guidelines used for varieties developed from MON 87705 will be consistent with those that growers of Monsanto's products are contractually obligated to abide by through the Monsanto Technology Stewardship Agreement (MTSA). Each soybean grower who will purchase a variety developed from MON 87705, or any other variety containing the Roundup Ready trait, must enter into a limited use license with Monsanto and must sign and comply with the MTSA. The MTSA incorporates by reference and requires the grower to follow Monsanto's Technology Use Guide (TUG), which sets forth the requirements and best practices for the cultivation of crops with the Roundup Ready trait including recommendations on weed-resistance management practices. Among each grower's legal commitments is the federal

requirement to use all Roundup herbicides in accordance with the directions for use on their labels, which incorporate weed resistance management practices. These are inclusive of the weed resistance management guidelines that are found on the EPA approved Roundup agricultural herbicide labels.

The weed-resistance management practices articulated in the TUG are broadly communicated to growers and retailers in order to minimize the potential for the development of glyphosate-resistant weeds. These practices are communicated through a variety of means, including direct mailings to each grower purchasing a Roundup Ready crop product, a public website (www.weedresistancemanagement.com), and reports in farm media publications. The overall stewardship program is reinforced through collaborations with U.S. academics who provide their recommendations for appropriate stewardship of the use of Roundup agricultural herbicides with Roundup Ready crops, as well as by crop commodity groups who have launched several weed-resistance educational modules available on their websites. Finally, Monsanto urges farmers to report any incidence of repeated nonperformance of Roundup agricultural herbicides on a particular weed, and Monsanto investigates cases of unsatisfactory weed control to determine the cause as defined in Section L-10 of this Appendix.

In cases where resistance is confirmed, Monsanto and University/CES provide recommendations for alternative control methods for farmers (see Table L-2). These recommendations are made available through Monsanto supplemental labels, Monsanto TUG, Monsanto and University publications and internet sites to growers, consultants, retailers and distributors. In all cases of glyphosate-resistant weeds in the U.S. and globally, there are alternative herbicides and cultural methods available to farmers to effectively control these species. Some examples of these recommendations from University/CES personnel are found in Table L-2. It is important to note that there are many alternative options in each situation.

Table L-2. Management Reccomendations for Control of Glyphosate Resistant Weeds

Glyphosate Resistant Weed	**State**	**Crop**	**Recommendations for alternative herbicides to manage glyphosate resistant weeds[1]**	**Reference** (Bulletin No.)
Palmer amaranth	AR	Soybean	Burndown: flumioxazin and Pre: flumioxazin or metolachlor, and/or Post: fomesafin	U of AR (FSA2152) www.uaex.edu
	AR	Cotton	PPI: triflualin or pendimethalin and/or Pre: diuron or fluometuron and/or E. Post: metolachlor and/or Post directed: diuron or prometryn or Layby: flumioxazin	U of AR (FSA2152) www.uaex.edu
Waterhemp	MO	Corn	Pre: metolachlor or acetachlor or isoxaflutole or mesotrione or atrazine, and/or Post: atrazine or dicamba or 2,4D	U of MO (IPM1030) www.extension.missouri.edu
	MO	Soybean	Pre: metribuzine or sulfentrazone or metolachlor or flumioxazin and/or Post: lactofen or fomesafen or aciflorfen	U of MO (IPM1030) www.extension.missouri.edu
Common ragweed	OH / IN	Corn	Pre: atrazine or dicamba or acetochlor and/or Post: dicamba or tembotrione or mesotrione, or troprmezone	2009 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience
	OH / IN	Soybean	Burndown: 2,4D and Pre: metribuzine or flumioxazin or cloransulam and/or Post: cloransulam or fomesafen or lactofen	2009 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience
Giant ragweed	OH / IN	Corn	Burndown: 2,4D +atrazine and Pre: Lumax or atrazine+isoxaflutole and/or Post: atrazine or dicamba or tembotrione or mesotrione, or troprmezone	2009 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience

Table L-2 (cont.). Management Reccomendations for Control of Glyphosate Resistant Weeds

Glyphosate Resistant Weed	**State**	**Crop**	**Recommendations for alternative herbicides to manage glyphosate resistant weeds[1]**	**Reference** (Bulletin No.)
Giant ragweed	OH / IN	Soybean	Burndown: 2,4D and Pre: Canopy or Envive or imazaquin or Authority or flumioxazin or cloransulam and/or Post: cloransulam or fomesafen or lactofen+bentazon	2009 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience
Marestail	TN	Corn	Burndown: 2,4D or dicamba or Pre: atrazine and/or Post: dicamba	2009 TN Weed Control Guide (PB1580) www.weeds.utk.edu
		Soybean	Burndown: 2,4D or dicamba or flumioxizin or Pre: metribuzin or fluioxazin and/or Post:cloransulam	2009 TN Weed Control Guide (PB1580) www.weeds.utk.edu
		Cotton	Burndown: dicamba or flumioxazin or trifloxysulfuron or Pre: fluometuron or diron or prometryn and/or Post:trifloxysulfuron and/or Post-directed: flometuron+MSMA or diuron+MSMA or prometryn+trifloxysulfuron	2009 TN Weed Control Guide (PB1580) www.weeds.utk.edu

[1](Burndown=before planting; Pre= preemergence; Post= postemergence; Post-directed= applied postemergence directed at the base of the crop; PPI=Pre Plant Incorporated)

L.10 Monsanto Weed Performance Evaluation and Weed Resistance Management Plan

To support and enhance Monsanto's weed management principles, stewardship program, and grower recommendations, Monsanto implements a Weed Performance Evaluation Program (WPEP) based on grower performance inquiries and field trial observations. In addition, a Monsanto Resistance Management Plan (MRMP) will be implemented as warranted. Each of these is discussed below.

The goal of the WPEP program is to continue to adapt, modify, and improve Monsanto's weed control recommendations to growers, with a focus on: (a) identifying performance issues with particular weeds and growing conditions; (b) providing product support to growers who are not satisfied with their level of weed control; and (c) identifying and investigating potential cases of glyphosate resistance early so that mitigation strategies can be implemented.

The WPEP is initiated when a grower reports to Monsanto, a retailer and/or University/CES personnel instances of unsatisfactory weed control following a glyphosate application. Follow-up with the grower is initiated by any of the three parties listed above to understand the situation, understand the reason for the inquiry and resolve it to the satisfaction of the customer. By virtue of Monsanto's presence in the marketplace, in cases where the initial contact with the grower is a retailer or University/CES person, Monsanto will be contacted and will engage in the technical investigation. It is important to Monsanto, as part of its proactive stewardship commitment, that these product performance inquiries are acted upon and investigated quickly.

The vast majority of these reported herbicide performance issues are determined to be due to application error or environmental conditions and are resolved through a phone conversation with the grower. However, a system is in place to investigate isolated or recurring performance problems for a weed species in a specific field. As warranted, a Monsanto, retailer or University/CES technical representative will arrange an on-site visit. If resistance is suspected to be a cause of the performance problem and has not already been confirmed for the specific weed and/or geography, additional steps will be taken to further explore the possibility of a new resistant weed species or population. These are the first steps of the Monsanto Resistance Management Plan (MRMP). When the reported problem involves a weed where resistance is common in the geography, the technical representative may simply confirm that the grower is managing a resistant population in an appropriate manner.

The MRMP consists of two primary elements: (a) initiation of testing steps to verify cases of suspected resistance, and (b) development and communication of Monsanto and/or University/CES guidelines to incorporate resistance mitigation into weed management programs where resistance is confirmed.

As part of the steps to verify cases of suspected resistance and if the on-site technical investigation cannot rule out potential resistance, seed may be collected from the subject site for follow-up greenhouse trials at Monsanto or at another University or third-party testing facilities that are set up to do this type of evaluation. If greenhouse trials do not repeat the observation and the weed is clearly controlled at label rates, then a thorough follow-up visit is conducted with the grower to re-evaluate the application recommendations and conditions of the operation that may be impacting weed control. If the greenhouse efficacy trials do

indicate insufficient control at label rates, then additional studies may be conducted to verify if the weed is resistant or, if the Monsanto, University/CES or third-party researcher has sufficient evidence, resistance may be considered to be confirmed without further testing. Resistance is considered to be confirmed if the two resistance criteria outlined in the Weed Science Society of America (www.wssa.net) (described in section L.3) are deemed to be fulfilled either through field and/or greenhouse data.

When resistance is confirmed, the scientific and grower communities are notified and a weed resistance mitigation plan is implemented by Monsanto in cooperation with the University/CES. The mitigation plan is designed to manage the resistant biotype through effective and economical weed management recommendations implemented by the grower. The scope and level of intensity of the mitigation plan may vary depending on a combination of the following factors: (a) biology and field characteristics of the weed (seed shed, seed dormancy, etc.), (b) importance of the weed in the agricultural system, (c) resistance status of the weed to other herbicides with alternate modes of action, and (d) availability of alternative control options. These factors are analyzed by Monsanto and University/CES personnel in combination with economic and practical management considerations to develop a tailored mitigation strategy. The plan considers what is technically appropriate for the particular weed and incorporates practical management strategies that can be implemented by the grower.

After a mitigation plan is developed, Monsanto communicates the plan to the grower community through the use of supplemental labeling (labeling which includes newly approved uses, use directions, or other instructions which have been added since the last EPA-approved Master label), informational fact sheets, retailer training programs, agriculture media and/or other means, as appropriate.

The final step of the MRMP may include extensive genetic, biochemical or physiological analyses of confirmed cases of glyphosate resistant weeds in order to elucidate the mechanism of resistance. The research findings are communicated to the scientific community through meetings and publications, and information pertinent to field applications is incorporated into weed management recommendations.

In addition to the grower inquiry initiated process, Monsanto, alone and in cooperation with University/CES, conducts field studies to understand the potential for weed resistance and weed shifts as the result of various weed management programs implemented in a Roundup Ready crop. These studies allow researchers to better track specific factors that can influence the development of resistance to specific weeds.

L.11 Summary

Development of weed resistance is a complex process that can be difficult to accurately predict. No single agronomic practice will mitigate resistance for all herbicides or all weeds. As a result, weed resistance needs to be managed on a case-by-case basis and tailored for the particular herbicide and weed in order to meet grower needs. Using good weed management principles, built upon achieving high levels of control through proper application rate, choice of cultural practices, and appropriate companion weed control tools will allow Roundup agricultural herbicides to continue to be used effectively. In cases where weed populations have developed resistance to glyphosate, effective management options are available and

experience has shown that growers continue to find value in using glyphosate in their weed control programs.

The key principles for effective stewardship of glyphosate use, including Roundup Ready crops, include: (1) basing weed management and weed resistance management practices on local needs and using the tools necessary to optimize crop yield, (2) using proper rate and timing of application, (3) not relying solely on one herbicide weed control option across a cropping system, (4) responding rapidly to instances of unsatisfactory weed control, and (5) providing up to date weed management and weed resistance management training.

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Appendix M. Nutritional Impact Evaluation of MON 87705 Soybean Oil

M.1 Intended Food Uses of MON 87705 Soybean Oil

It is anticipated that MON 87705 soybean oil would substitute for the liquid soybean oil in the market place. The use of liquid soybean oil is mainly in salad dressings, mayonnaise and spreads, home use liquid soybean oil, and margarines (stick, tub, light variety). These four food categories represent approximately 94% (5,498 million pounds) of the replaceable liquid soybean oil market in the U.S. (HyQuest, 2009).

In order to assess the nutritional impact from the introduction of MON 87705 into the marketplace, a dietary replacement model was used. In this model, MON 87705 soybean oil was substituted for the liquid soybean oil fraction in a subset of foods in the U.S. diet, including salad dressings, mayonnaise and spreads, home use liquid soybean oil, and margarines. This assessment assumed that all of the targeted oil components of the foods proposed for replacement at all eating occasions in the U.S. are replaced with MON 87705 soybean oil. The results presented in this assessment represent a conservative estimation of the effect of MON 87705 soybean oil on fatty acid (FA) composition of the diet. The assessment is exclusive of frying applications, which generally utilize hydrogenated or low linolenic oils. Fatty acid intake estimates in this dietary replacement model were prepared for Monsanto by Exponent (Washington D.C.)

A baseline FA composition for the soybean oil component of the four target food categories (mentioned above) was created using a combination of published FA compositions of liquid and partially hydrogenated soybean oils (ISEO, 2006; Exler et al., 1993), and market research analysis on currently utilized oil blends for the target foods.

M.2 Estimated Dietary Intake of MON 87705 Soybean Oil based on Proposed Food Uses

Four major sources of data were used to conduct the analysis: (1) Food intake data and nutrient composition from National Health and Nutrition Examination Survey (NHANES, CDC, 2007) 2003-2004 and 2005-2006, (2) Target foods and associated oils and Exponent's proprietary recipes, (3) Baseline fatty acid profile for the oils associated with target foods, and (4) Fatty acid profile for MON 87705 soybean oil. Exponent's proprietary software package, FARE™ version 8.43 was used to implement the analysis. The fatty acid composition of MON 87705 used in the dietary replacement model is summarized in Table M-1.

Table M-1. Fatty Acid Profile for MON 87705 Used in the Intake Estimations

Fatty Acids	Composition (% wt of Total FA)
16:0	2.4
18:0	3.3
18:1	76.5
18:2	10.1
18:3	6.7

Substitutions of MON 87705 soybean oil were done at the 100% substitution level, i.e., assuming 100% of the liquid soybean oil contained in these foods was replaced with the MON 87705 soybean oil, and every eating occasion of a target food was replaced by a comparable food containing MON 87705 soybean oil.

M.3 Estimated Daily Intake of Total Fat and Selected Fatty Acids before and after Replacement of Conventional Soybean Oil with MON 87705 Soybean Oil in Targeted Foods

The mean and 90th percentile intakes of total fat and five selected FA from conventional soybean (prior to substitution with MON 87705 soybean oil) were estimated for the target foods, and are reported in Tables M-2 and M-3. For the U.S.population, the soybean oil component of the target foods contributes an average of 4.27 g/day per capita of total fat; this amounts to approximately 5.4% of total fat from the whole diet. At the 90th percentile, per user, the total fat contribution from the soybean oil component of the target foods is 16.28 g/day, or approximately 12.6% of total fat from the whole diet. To normalize consumption of nutrients for the range of caloric intake across a population, percent of energy/caloric intake (% en) is used (IOM, 2002). Therefore, on a percent energy (% en) basis, the mean per capita contribution from the liquid soybean oil component of the target foods is 1.8% and the 90th percentile per user is 6.83%.

In MON 87705 soybean oil, intake of 18:1 oleic acid is increased the most among the five fatty acids evaluated. For the U.S. population, the mean per capita intake of 18:1 oleic acid from the MON 87705 soybean oil component from proposed foods increased from 1.04 g/day to 3.13 g/day (1.35 % en). The 90th percentile per user intake of 18:1 oleic acid increased from 3.86 g/day to 12.22 g/day (Tables M-2 and M-3).

Table M-2. Mean Per Capita Fatty Acid Intake from Target Foods Pre and Post MON 87705 Replacement, U.S.population

Fatty Acids	g/day			% En		
	Pre replacement	Post replacement	% change	Pre replacement	Post replacement	% change
Palmitic 16:0	0.45	0.13	-71.11	0.19	0.06	-68.42
Stearic 18:0	0.18	0.19	5.56	0.08	0.08	0.00
Oleic 18:1	1.04	3.13	200.96	0.45	1.35	200.00
Linoleic 18:2	1.93	0.39	-79.79	0.83	0.17	-79.52
Linolenic 18:3	0.25	0.26	4.00	0.11	0.11	0.00
Total Fat Intake	4.27	4.27	0.00	1.84	1.84	0.00

Table M-3. 90th Percentile Per User Fatty Acid Intake from Target foods, Pre and Post MON 87705 Replacement, U.S.Population

Fatty Acids	g/day			% En		
	Pre replacement	Post replacement	% change	Pre replacement	Post replacement	% change
Palmitic 16:0	1.71	0.48	-71.93	0.72	0.21	-70.83
Stearic 18:0	0.66	0.7	6.06	0.27	0.31	14.81
Oleic 18:1	3.86	12.22	216.58	1.64	5.12	212.20
Linoleic 18:2	7.9	1.58	-80.00	3.26	0.66	-79.75
Linolenic 18:3	1.03	1.05	1.84	0.42	0.44	4.76
Total Fat Intake	16.28	16.28	0.000	6.83	6.83	0.00

M.4 Impact of Replacement on Total Diet

The impact of oil replacement under the proposed food uses with MON 87705 soybean oil on the total daily intake (g/day and % en) of total fat and the five major fatty acids (palmitic, stearic, oleic, linoleic, linolenic) in the diet of the U.S. population are summarized in Tables M-4 and M-5. As expected, the replacement with MON 87705 soybean oil does not change the total daily fat intake, which remains at 79.58 g/day on the mean per capita basis and at 129.4 g/day in the 90th percentile per user group.

Table M-4. Fatty Acid Intake from Total Diet, Pre and Post MON 87705, U.S.Population (g/day)

Fatty Acids	Mean Per Capita			90th Percentile Per User		
	Pre MON 87705 (g/day)	Post MON 87705 (g/day)	% change	Pre MON 87705 (g/day)	Post MON 87705 (g/day)	% change
Palmitic 16:0	14.38	13.83	-3.80	23.62	21.48	-9.00
Stearic 18:0	6.96	6.98	0.30	11.75	11.84	0.80
Oleic 18:1	27.34	30.29	10.80	45.20	57.52	27.20
Linoleic 18:2	14.75	14.12	-4.30	25.66	23.08	-10.10
Linolenic 18:3	1.42	1.42	0.00	2.49	2.50	0.20
Total Fat Intake	79.58	79.58	0.00	129.40	129.40	0.00

Table M-5. Fatty Acid Intake from Total Diet, Pre and Post MON 87705, U.S.Population (% en)

Fatty Acids	Mean Per Capita			90th Percentile Per User		
	Pre MON 87705 (% en)	Post MON 87705 (% en)	% change	Pre MON 87705 (% en)	Post MON 87705 (% en)	% change
Palmitic 16:0	6.07	5.84	-3.70	8.00	7.10	-11.30
Stearic 18:0	2.91	2.91	0.00	3.94	4.03	2.40
Oleic 18:1	11.49	12.75	10.90	15.16	20.28	33.80
Linoleic 18:2	6.23	5.96	-4.40	9.22	8.05	-12.70
Linolenic 18:3	0.61	0.61	0.00	0.95	0.96	0.80
Total Fat Intake	33.61	33.61	0.00	42.88	42.88	0.00

Table M-6. Mean Per Capita and 90th Percentile Per User Fatty Acid Intake from Total Diet, Post MON 87705, Age and Gender Groups (g/day)

Population		Total Fat	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
U.S.	Mean	79.58	13.83	6.98	30.29	14.12	1.42
	90th percentile	129.40	21.48	11.84	57.52	23.08	2.50
Males 1-8	Mean	64.76	12.09	5.95	23.07	10.48	1.00
	90th percentile	100.53	17.73	9.40	38.23	16.54	1.63
Males 9-19	Mean	93.45	16.95	8.54	34.47	15.87	1.46
	90th percentile	142.14	24.38	13.45	61.62	24.93	2.48
Males 20-49	Mean	102.91	18.08	9.10	39.27	18.02	1.77
	90th percentile	155.60	26.78	14.44	68.31	27.48	2.97
Males 50+	Mean	87.93	14.82	7.56	34.33	15.92	1.64
	90th percentile	139.74	22.57	12.27	62.39	26.17	2.83
Females 1-8	Mean	60.85	11.36	5.62	21.84	9.80	0.97
	90th percentile	91.46	16.47	8.71	36.82	15.75	1.55
Females 9-19	Mean	72.86	12.96	6.57	27.22	12.92	1.24
	90th percentile	111.56	18.71	10.24	48.62	20.25	2.08
Females 20-49	Mean	73.65	12.57	6.39	28.27	13.40	1.39
	90th percentile	114.17	18.48	10.26	52.51	20.70	2.35
Females 50+	Mean	63.30	10.43	5.38	24.76	11.80	1.31
	90th percentile	100.54	15.75	8.75	45.77	19.21	2.29

Following replacement with MON 87705 soybean oil, there is an overall increase in the total daily intake of 18:1 oleic acid from 27.34 g/day (11.49 % en) to 30.29 g/day (12.75 % en) on a mean per capita basis and an increase from 45.2 g/day (15.16 % en) to 57.52 g/day (20.28 % en) at the 90th percentile per user (Tables M-4 and M-5). These increases post-replacement are expected, since the 18:1 oleic acid fraction in conventional soybean oil is much lower compared to the 76.5% oleic acid observed in MON 87705. The pattern of total daily intake of fatty acids in sub groups stratified by age and gender are similar to that of the overall U.S. population, with 18:1 oleic acid having the largest increase following replacement of conventional soybean oil with MON 87705 soybean oil (Table M-6). The Dietary Guidelines for Americans Technical Report (USHHS, 2005) stated that MUFAs are not required in the diet; however, they provide a vehicle to achieving total fat intake recommendations within the context of saturated fat and PUFA recommendations. Further, substitution of oleic acid for saturated fat in the diet can reduce low density lipoprotein cholesterol (LDL-C).

Total daily intakes of 16:0 palmitic acid decreased as a consequence of dietary replacement with MON 87705 soybean oil (Tables M-4 and M-5). On a mean per capita basis, 16:0 palmitic acid intake decreased from 14.38 g/day (6.07 % en) to 13.83 g/day (5.84 % en), while the decrease was predictably greater in the per user 90th percentile group, where daily intake decreased from 23.62 g/day (8.00 % en) to 21.48 g/day (7.10 % en) (Table M-6). Total dietary intakes of 18:0 stearic acid were similar in pre- and post- MON 87705 replacement analysis, in both mean per capita (6.96 g/day and 6.98 g/day, respectively) and 90th percentile per user groups (11.75 g/day and 11.84 g/day), respectively. A decrease in dietary saturated fat intake,

as shown here for 16:0 palmitic acid, is consistent with current dietary recommendations (Lichtenstein et al., 2006; USDA-ERS, 2005; WHO/FAO, 2003).

Linoleic acid (18:2) intake levels decreased by a similar rate, from 14.75 g/day (6.23 % en) to 14.12 g/day (5.96 % en) on a mean per capita basis; and from 25.66 g/day (9.22 % en) to 23.08 g/day (8.05 % en) in the 90th percentile per user group. An adequate intake (AI) for 18:2 linoleic acid of 17 g/day for men (19-50 years) and 12 g/day for women (19-50 years) has been established by the Institute of Medicine (IOM, 2002). The values observed post-replacement for the respective subgroups are consistent with these recommendations. For example, post replacement values are 18.02 g/day (males 20-49) and 13.40 g/day (females 20-49) (Table VII-8). Further, the population and adult subgroup 18:2 linoleic intakes post replacement are still in line with U.S. Dietary Guidelines (USHHS, 2005) and the Acceptable Macronutrient Distribution Range of 5-10% energy (IOM, 2002), which is designed to reduce risk for chronic disease. For example, the population mean intake of linoleic acid was 6.23% energy before replacement and 5.96% energy post-replacement (Table M-5).

There was no change in 18:3 linolenic acid after replacement with MON 87705 oil (Table M-4, M-5). An adequate intake (AI) for 18:3 linolenic acid of 1.6 g/day for men (19-50 years) and 1.1 g/day for women (19-50 years) has been established by the Institute of Medicine (IOM, 2002). The values observed both pre and post replacement for the respective subgroups are consistent with these recommendations. For example, post replacement values are 1.77 g/day (males 20-49) and 1.39 g/day (females 20-49) (Table M-6).

In summary, replacement of conventional soybean oil with MON 87705 soybean oil under the proposed food uses results in changes in the fatty acid composition in the U.S. diet that lead to higher oleic acid intake, and lower consumption of saturated fats (i.e., 16:0 palmitic) and linoleic acid, with no impact on total fat intake. Given that this assessment assumes that all of the targeted oil components of the foods proposed for replacement at all eating occasions in the U.S. are replaced with MON 87705 soybean oil, the results presented here represent a theoretical maximal effect of MON 87705 on fatty acid composition of the diet. The nutritional impact of exposure to MON 87705 soybean oil in targeted foods under the intended conditions of use is estimated to result in changes in fatty acid consumption that are in line with current dietary guidelines for fatty acid intake (Lichtenstein et al., 2006; USDA-ERS, 2005; WHO/FAO, 2003).

M.5 Composition and Nutrition Assessment Conclusion

In conclusion, the nutritional impact from the use of MON 87705 soybean oil in targeted foods under the intended conditions of use, is estimated to result in changes in fatty acid consumption that are in line with current dietary guidelines for fatty acid intake. Therefore, MON 87705 is regarded to be as safe and nutritious as conventional soybean for food use.

Appendix M References

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Appendix N. Glyphosate-Tolerant Crops: Potential Impacts on Land Use and Agricultural Practices

N.1 Introduction

Since 1994, USDA has granted nonregulated status to 13 glyphosate-tolerant biotechnology-derived products (Table N-1). Petitions for three additional glyphosate-tolerant products have been submitted to the agency, with deregulation decisions pending for alfalfa, corn and creeping bentgrass. The crops for which petitions have been approved or are pending include: corn, cotton, creeping bentgrass, rapeseed (canola), soybean, and sugar beet. This document discusses the cumulative impacts on land use and agricultural practices from deregulation of glyphosate-tolerant crop products, considering the existing glyphosate-tolerant crops and those potentially available in the reasonably foreseeable future based on pending petitions.

N.2 Impact on Land Use

USDA has granted nonregulated status to events conveying the glyphosate-tolerance trait in five different crops (Table N-1). These crops have been rapidly adopted by U.S. farmers and since their introduction have been grown cumulatively on more than 778 million acres. In 2007 alone, glyphosate-tolerant crops were planted on approximately 120 million acres in the U.S.[11] The sections below assess the impact of the adoption of glyphosate-tolerant crops on cropping patterns.

Table N-1. Herbicide-Tolerant Crops Undergoing Review or Previously Deregulated by USDA-APHIS.

Petition	Crop Species	Trait(s)	Status
93-258-01p	Soybean	Glyphosate-tolerant	Deregulated
95-045-01p	Cotton	Glyphosate-tolerant	Deregulated
96-317-01p	Corn	Glyphosate-tolerant and Corn Borer-resistant	Deregulated
97-099-01p	Corn	Glyphosate-tolerant	Deregulated
98-173-01p	Sugar Beet	Glyphosate-tolerant	Deregulated
98-216-01p	Canola	Glyphosate-tolerant	Deregulated
00-011-01p	Corn	Glyphosate-tolerant	Deregulated
01-324-01p	Canola	Glyphosate-tolerant	Deregulated
03-104-01p	Creeping Bentgrass	Glyphosate-tolerant	Pending
03-323-01p	Sugar Beet	Glyphosate-tolerant	Deregulated
04-086-01p	Cotton	Glyphosate-tolerant	Deregulated
04-110-01	Alfalfa	Glyphosate-tolerant	Pending
06-178-01p	Soybean	Glyphosate-tolerant	Deregulated
06-271-01p	Soybean	Glyphosate- and Acetolactate Synthase Inhibitor-tolerant	Deregulated
06-332-01p	Cotton	Glyphosate-tolerant	Deregulated
07-152-01p	Corn	Glyphosate- and Imidazolinone-tolerant	Pending

[11] Calculated from datasets found at www.ers.usda.gov/data/biotechcrops.

N.3 Total Cropland Acres

The overall U.S. area planted to principal crops, which include corn, sorghum, oats, barley, winter wheat, rye, durum, 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 at 326 million acres since the introduction of glyphosate-tolerant crops in 1996[12]. If fact, the total U.S. cropland acres have been relatively constant since the 1940s (Vesterby and Krupa, 1997). Therefore, the introduction of glyphosate-tolerant crops has not resulted in a significant change to the total cropland acres in the U.S.

Following deregulation and commercial introduction, the acres of glyphosate-tolerant varieties/hybrids planted for each crop has steadily increased (see Figures N-1 through N-3 below). However, rapid adoption of glyphosate-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. The impact of glyphosate-tolerant crop deregulation or pending deregulation and adoption on crop acres is discussed below.

N.3.1 Canola

Glyphosate-tolerant canola was commercialized in the U.S. in 1999. In 2007, U.S. growers planted 1.2 million acres of canola (USDA-NASS, 2007) and of those acres, 0.61 million acres, or approximately 50%, contained the glyphosate-tolerance trait (Monsanto, 2007).

The total canola acreage since 1990 and the acreage of glyphosate-tolerant canola since commercialization in 1999 are provided in Figure N-1. The total U.S. acreage of canola increased 200% from 367,000 acres in 1996 to 1.1 million acres in 1998, coinciding with passage of the 1996 Federal Agriculture Improvement Act.[13] This acreage peaked at 1.55 million in 2000 and has remained near 1.l million acres since 2005.

The acreage of glyphosate-tolerant canola increased rapidly following commercial introduction in 1999 and has remained between 50 and 70% of planted canola acres since 2001. The dramatic fluctuation in total canola acreage before and after glyphosate-tolerant canola was commercialized indicates that factors unrelated to the availability of the glyphosate-tolerant trait play a larger role in acres planted than the availability of the glyphosate-tolerant trait.

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[12] Calculated from datasets at http://usda.mannlib.cornell.edu/usda/current/htrcp/ by comparing the total acres from 1983 to 1995 and from 1996 to 2007.

[13] The 1996 Federal Agriculture Improvement Act, 7 U.S.C. § 7201 *et seq.*, gave growers almost complete flexibility in selecting the crops they could plant.

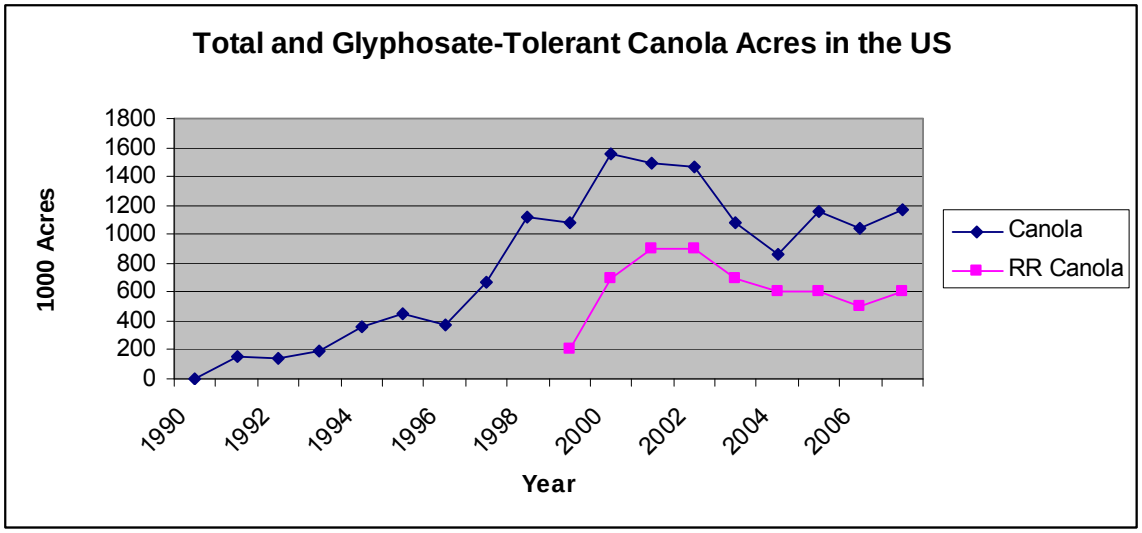

Figure N-1. Total Acres of Canola Relative to Acres of Glyphosate-tolerant Varieties planted. Data obtained from http://usda.mannlib.cornell.edu/usda/current/htrcp/ for total canola acres and www.monsanto.com/pdf/investors/2007/FY2007BiotechAcres.pdf for glyphosate-tolerant canola acres.

N.3.2 Corn

Glyphosate-tolerant corn was commercialized in the U.S. in 1996. From 1996 to 2007, glyphosate-tolerant corn hybrids were grown on 151 million cumulative acres. In 2007, U.S. growers planted 93.6 million acres of field corn (USDA-NASS, 2007); 48 million acres, or 52%, contained a glyphosate-tolerance trait (USDA- ERS, 2007).

Since its introduction, the percentage of glyphosate-tolerant corn has increased steadily to 52% in 2007. The total corn acreage, however, remained relatively steady from 1996 to 2006, with a yearly average of 78 million acres (see Figure N-2), indicating that the glyphosate-tolerant trait has had little impact on overall corn acreage. In 2007, the total corn acreage was up 15% from 2006 to 92.9 million acres. This increase was attributed to increased demand from ethanol producers and strong exports sales[14]. The increase in total corn acres, including an increase in glyphosate-tolerant corn acres, resulted in fewer acres of soybean planted in the Corn Belt and Great Plains and fewer acres of cotton and rice planted in the Delta and Southeast regions.

[14] http://www.usda.gov/nass/PUBS/TODAYRPT/acrg0607.txt

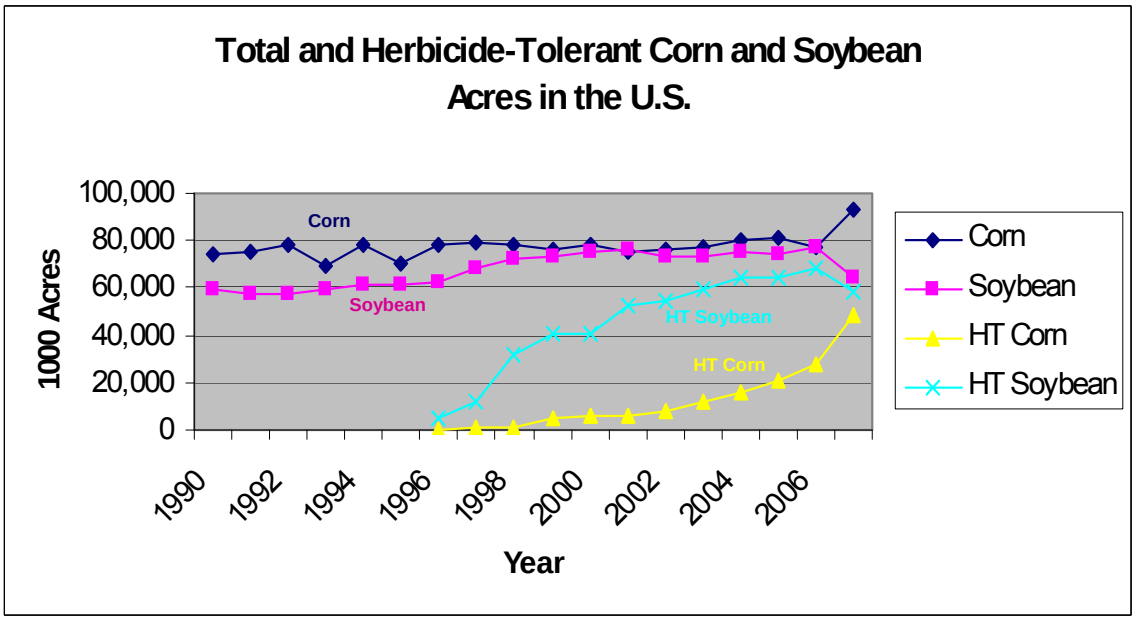

Figure N-2. Total Acres of Corn and Soybean Relative to Acres of Herbicide-tolerant (HT) Varieties Planted. The varieties are predominately glyphosate-tolerant varieties, but also include phosphinothricin-tolerant hybrids for corn. Data obtained from http://usda.mannlib.cornell.edu/usda/current/htrcp/ for total crop acres and USDA-ERS (2007) for herbicide-tolerant crops acres.

N.3.3 Soybean

Glyphosate-tolerant soybean was commercialized in the U.S. in 1996. From 1996 to 2007, glyphosate-tolerant soybean was grown on more than 509 million cumulative acres. In 2007, growers planted 64 million acres of soybean in the U.S. (USDA-NASS, 2007); 58 million acres, or 91%, contained the glyphosate-tolerance trait (USDA-ERS, 2007).

Since its introduction, the percentage of glyphosate-tolerant soybean has increased steadily to 91% in 2007 (Figure 2). The total acres of soybean increased 23% (62.4 to 76.9 million acres) from 1996 to 2006. This increase was due to higher global demand, but was facilitated by increased planting flexibility from: (1) the 2002 Farm Bill; (2) steadily rising yield improvements from narrow-rowed seeding practices; (3) a greater number of 50-50 corn-soybean rotations; and (4) lower production costs, partly due to widespread adoption of herbicide-tolerant varieties (Ash et al., 2006). In 2007, acres of soybean were down 17% to 64.1 million compared to 2006 due to growers planting more corn, as discussed above.

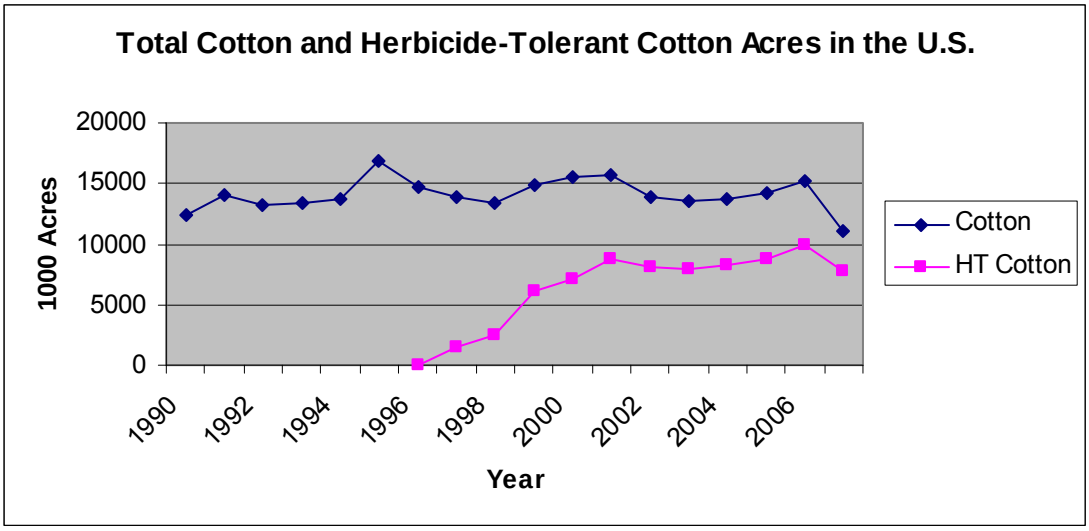

Figure N-3. Total Acres of Cotton Relative to Acres of Herbicide-tolerant (HT) Varieties Planted. The varieties are predominately glyphosate-tolerant varieties, but also include phosphinothricin-tolerant varieties. Data obtained from http://usda.mannlib.cornell.edu/usda/current/htrcp/ for total crop acres and USDA-ERS (2007) for herbicide-tolerant crops acres.

N.3.4 Cotton

Glyphosate-tolerant cotton was commercialized in the U.S. in 1997. From 1997 to 2007, glyphosate-tolerant cotton was grown on more than 76 million cumulative acres. In 2007, growers planted 11 million acres of cotton in the U.S. (USDA-NASS, 2007) of which 7.7 million acres, or 70%, contained a glyphosate-tolerance trait (USDA-ERS, 2007).

Since its introduction, the percentage of glyphosate-tolerant cotton increased to approximately 60% in 2001 and remained near that level until reaching 70% in 2007 (Figure N-3). The total cotton acreage, however, remained relatively constant from 1997 to 2006 at 14.1 million +/- 1.3 million acres (Figure N-3). In 2007, the total cotton acres were down 28% due to a shift to corn and soybean across the Southeast U.S.[15]

N.3.5 Sugar beet

Since 1990, the total U.S. sugar beet acreage has remained relatively constant at 1.4 million acres (USDA-NASS, 2007). In 2007, approximately 1.3 million acres of sugar beet were planted in the 11 largest sugar beet production states in the U.S. Glyphosate-tolerant sugar beet has only been grown on limited acres since its deregulation in 2005, but is expected to be grown on substantially more acres in 2008. Regardless, the acres forecasted by USDA for sugar beet in 2008 are 1.08 million acres[16], down slightly from 1.3 million acres in 2007, again due to the market factors described above.

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[15] http://www.usda.gov/nass/PUBS/TODAYRPT/acrg0607.txt
[16] http://usda.mannlib.cornell.edu/usda/current/Acre/

N.3.6 Bentgrass

Creeping bentgrass is widely grown on about 77,000 acres on golf course putting greens, tees, and fairways in the cooler climates throughout the U.S., and exclusively for greens in the south where specialized root zone management systems allow survival of creeping bentgrass under summer heat conditions. After deregulation, glyphosate-tolerant creeping bentgrass is expected to replace conventional creeping bentgrass on some golf courses. The glyphosate-tolerance trait does not alter the overall biology and ecology of creeping bentgrass[17] therefore, glyphosate-tolerant creeping bentgrass is not expected to impact land use any differently than conventional creeping bentgrass.

N.3.7 Alfalfa

Alfalfa grown for forage is a major crop in the U.S. More than 20 million acres have been planted annually since 1950. Acreage peaked in the mid-1960s at approximately 30 million acres and declined gradually to approximately 21 million acres in 2007 (USDA-NASS 2007), in part due to increased use of corn silage as a forage.

Data from the previously deregulated glyphosate-tolerant crops shows that market forces and governments programs (1996 and 2002 Farm Bills), not the presence of glyphosate-tolerant crop varieties, are primarily responsible for any significant changes in crop acreage. Based on the adoption rates of other glyphosate-tolerant crops, it is expected that the deregulation and commercial availability of glyphosate-tolerant alfalfa would replace a significant portion of conventional alfalfa varieties. The availability of these glyphosate-tolerant varieties provides for more efficient weed control and thus allows growers more flexibility when choosing what crop varieties to plant on their farms. This may result in a shift back from corn silage to alfalfa hay for animal feed; thus increasing the alfalfa acreage to the level seen in the 1980s and 1990s. However, based on the experience with the other glyphosate-tolerant crops, deregulation of glyphosate-tolerant alfalfa is expected to have little impact on the overall acreage historically seen for alfalfa or other crops.

N.3.8 Conventional and Organic Production

Since the introduction of glyphosate-tolerant crops, the total acreage of principal crops and acreage of each specific crop have remained relatively constant, as discussed above. Therefore, the increase in acreage of glyphosate-tolerant crops comes at the expense of crop varieties used for conventional production, although conventional varieties are still widely available to those who choose to plant them (see Section XI.D.3). USDA data show that the acreage of organic corn and soybean has actually increased since the introduction of glyphosate-tolerant crops in 1996 (Figure N-4). The acreage of organic cotton has decreased from a high of 32,850 acres in 1995 to 9,537 acres in 2005, which is comparable to the acreage planted in 1993 and 1994 before the introduction of glyphosate-tolerant cotton. The decrease in acreage is attributed to increased market competition from imported organic cotton (USDA-ERS, 2006). Therefore, the use of glyphosate-tolerant varieties has replaced conventional

[17] http://www.aphis.usda.gov/brs/aphisdocs/03_10401p.pdf

varieties, resulting in a change in agricultural practices related to weed control and soil tillage, as discussed below. Based on adoption rates of other glyphosate-tolerant crops, it is expected that deregulation of glyphosate-tolerant alfalfa will result in a replacement of a significant percentage of conventional alfalfa with glyphosate-tolerant alfalfa. Less than one percent of alfalfa hay acres were harvested in 2005 as organic (USDA-NASS, 2006). Depending on market demand, and again based on the experiences of the corn and soybean markets, a similar increase in organic alfalfa acres is possible.

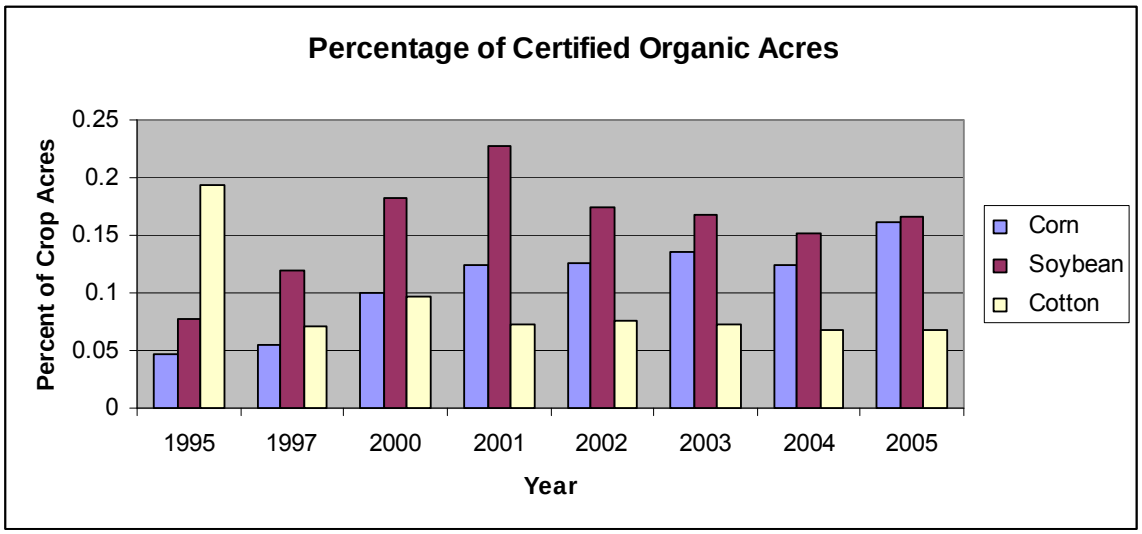

Figure N-4. Percentage of Certified Organic Acres for Corn, Cotton, and Soybean. Data obtained from www.ers.usda.gov/Data/organic and www.nass.usda.gov for total acres**.**

N.4 Impact on Herbicide Use and Costs

N.4.1 Canola

For a description of weed control practices in conventional canola, please see the recommendations from the North Dakota State University Extension Service[18].

For glyphosate-tolerant canola, it is recommended that glyphosate be applied at a maximum rate of 0.38 lb acid equivalent (ae)/acre (A) with no more than two applications to glyphosate-tolerant canola from emergence to bolting (Zollinger, 2008). On average, a typical weed management program in conventional canola (that could provide control comparable to the program in herbicide-tolerant canola) cost about $39/A in 2005. In contrast, weed-management costs in glyphosate-tolerant canola, inclusive of technology fee/seed premium, were about $24/A, for a total cost reduction of 62%. The net impact on gross margins has been an increase of between $47/ha and $55/ha for glyphosate-tolerant canola (Brooks and Barfoot, 2006).

In 2005 in North Dakota, which contained 92% of the total U.S. canola acreage, glyphosate-tolerant canola was planted on 676,000 acres. Use of the glyphosate-tolerant varieties resulted

[18]http://www.ndsu.edu/weeds/weed_control_guides/2009_weed_control_guide/

in reduction in total weed management costs by $10 million and a reduction in herbicide use by 426,000 total pounds (Sankula, 2006).

Therefore, glyphosate-tolerant canola provides significant cost savings to the growers and reduces the amount of herbicide used.

N.4.2 Corn

A description of weed control practices in conventional corn can be found in USDA petition 04-125-01p, p. 128[19].

A mixture of the herbicides registered for use in corn provides fair to excellent control of most weeds in a conventional weed control system. However, growers must know the specific weed problems present on their farms or fields to select the most effective herbicide program. Typical weed management in conventional corn includes a preemergence application of metalachlor+atrazine followed by a postemergence application of mesotrione plus a premix of nicosulfuron and rimsulfuron (Sankula, 2006).

Two major options are used for weed management in glyphosate-tolerant corn (Sankula, 2006). The first and most widely used option is the use of half-rate of metolachlor + atrazine as a preemergence herbicide, followed by glyphosate as a postemergence application. The second approach involves a total postemergence-based program with either one or two applications of glyphosate or tank-mix applications of glyphosate with atrazine.

Sankula (2006) compared weed management programs and costs associated with glyphosate-tolerant and conventional corn. Weed management costs in 2005 were 25% lower in glyphosate-tolerant corn compared to conventional corn. In 2005 alone, glyphosate-tolerant corn reduced the herbicide use in corn by 18.3 million pounds of active ingredient and reduced weed management costs by $238.2 million (Sankula, 2006).

Brookes and Barfoot (2006) compared a typical herbicide treatment regime for glyphosate-tolerant corn and conventional corn. They calculated the herbicide use rate and the Environmental Impact Quotient (EIQ), a measure of impact to the environment. The EIQ integrates the various environmental impacts of a single pesticide into a single field value per hectare. The EIQ value is then multiplied by the amount of pesticide used per hectare to get the overall EIQ rating. A lower EIQ rating indicates a lower impact on the environment.

The conventional herbicide program used a mix of herbicides, such as acetochlor, atrazine, primsulfuron, and dicamba. The average herbicide use rate by growers of conventional corn was 3.74 kg active ingredient (ai)/hectare (ha), with a field EIQ rating of 76.6/ha. The glyphosate-tolerant corn program used glyphosate plus lower rates of acetochlor and atrazine than those used on conventional crops. The values for the glyphosate-tolerant corn program included an average herbicide use rate of 2.59 kg ai/ha, with a field EIQ rating of 48.4/ha.

Based on these analyses, the glyphosate-tolerant corn program has a significantly better environmental profile than the conventional herbicide program.

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[19] http://www.aphis.usda.gov/brs/aphisdocs/04_12501p.pdf

N.4.3 Cotton

For a description of weed control practices in conventional cotton, please see USDA petition 04-086-01p[20].

For glyphosate-tolerant cotton, typical weed management programs consist of a preemergence application followed by postemergence applications of glyphosate or a glyphosate only system for pre- and postemergence applications. The overall impact of herbicide-tolerant cotton on U.S. agriculture in 2005 alone was a reduction in crop production costs of $39 million and pesticide use of 18 million pounds of active ingredient (Sankula, 2006).

According to Brooks and Barfoot (2006), the use of herbicide-tolerant cotton[21] (of which 98% was tolerant to glyphosate) led to reduced production costs and increased profitability from 1997 to 2005 of between $21/ha and $49/ha annually. The net gain to farm income in 2005 was $161 million. Since glyphosate-tolerant cotton was first commercially produced in 1997, the farm income benefit has been $919 million. In terms of added value, the effect on farm income in 2005 was equivalent to an increase in production of 3% (151,000 tons).

N.4.4 Soybean

A description of herbicide use in conventional soybean is available in USDA petition 06-178-01P[22].

Conventional herbicide programs typically require a preemergence application with one to two herbicides, followed by a postemergence application with one to two herbicides. Conversely, glyphosate-tolerant soybean typically require only one timely application of glyphosate at 0.95 lb ai/A. Consequently, in 2005, growers planting glyphosate-tolerant soybean varieties reduced the overall number of herbicide applications by 39.4 million, which translated to cost savings of $134 million (Sankula, 2006). On average, glyphosate-tolerant soybean programs used 1.03 lb ai/A at a cost of $21.28/A in 2005, compared to growers of conventional soybean varieties who used an additional 0.32 lb ai/A or 32% more herbicide active ingredients at an additional cost of $18.09/A. When considering herbicide costs and application costs in 2005 alone, U.S. soybean growers saved $1.17 billion on weed management costs using glyphosate based weed-control programs (Sankula, 2006).

This reduction of herbicide use and the physical properties of glyphosate have secondary environmental benefits also. Shipitalo, et al. (2008) investigated the transport loss of herbicides from agriculture fields due to runoff. They found that the use of glyphosate-tolerant soybean usually, but not always, resulted in substantially less runoff than the residual herbicides that glyphosate herbicides replaced. In contrast to the residual herbicides, the glyphosate concentrations from runoff in the watershed remained below the allowable limit for drinking water. The authors concluded that the impact of herbicide losses in runoff resulting from production of growing transgenic, glyphosate-tolerant soybean should be reduced (Shipitalo, 2008).

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[20] http://www.aphis.usda.gov/brs/aphisdocs/04_08601p.pdf

[21] 2% of the herbicide-tolerant cotton was glufosinate-tolerant cotton.

[22] http://www.aphis.usda.gov/brs/aphisdocs/06_17801p.pdf

N.4.5 Sugar beet

For a description of the weed control practices in conventional sugar beet, please see USDA petition 03-323-01p[23].

As of 2007, glyphosate-tolerant sugar beet did not have a history of widespread production, although a significant portion of the sugar beet acres is expected to be planted with glyphosate-tolerant varieties in 2009. USDA-NASS data are not yet available on adoption rates, impacts on herbicide use rates, and other relevant parameters, a predictive *ex ante* assessment of potential impact is included in Appendix A. Coyette et al. (2002) predicted that introduction of glyphosate-tolerance technology in sugar beet cultivation in Europe would lead to a reduction in herbicide use by as much as 43%. Based on this study and data from other glyphosate-tolerant crops, beneficial results in cost savings, pesticide reduction, and weed control are expected.

N.4.6 Bentgrass

Glyphosate-tolerant creeping bentgrass is currently under review for deregulation and APHIS is conducting an environmental impact statement (EIS) to support its decision-making under NEPA.

The stated rationale for developing glyphosate-tolerant creeping bentgrass event ASR368 was to benefit current agronomic practices in creeping bentgrass seed production and its use as a principal turf on golf course tees, greens, and fairways. Control and management of annual and perennial grass, broadleaf, and sedge weed species that invade golf courses has met with variable success using a variety of herbicides and plant growth regulators. A discussion of the pesticides used on conventional bentgrass is available in USDA petition 03-104-01p, p. 290[24]. The most troublesome of these weeds includes annual bluegrass, rough stalk bluegrass, and bermuda grass. Considerable investments in labor, chemicals, water, and time are made to manage creeping bentgrass in golf course fairways and putting greens invaded by annual bluegrass because of bentgrass' susceptibility to disease, heat, and drought stress. Grassy weeds occurring in creeping bentgrass seed production areas can reduce the purity of harvested seed, and can limit the land on which such seed production can profitably occur. Golf courses and seed producers who adopt glyphosate-tolerant creeping bentgrass instead of conventional creeping bentgrass varieties could use the Roundup family of industrial turf and ornamental herbicides as needed (pending U.S. Environmental Protection Agency label change approvals) as an effective, new, over-the-top method to control the majority of annual and perennial grasses and broadleaf weeds.

N.4.7 Alfalfa

For a description of the weed control practices in conventional alfalfa, please see USDA petition 04-110-01p[25].

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[23] http://www.aphis.usda.gov/brs/aphisdocs/03_32301p.pdf
[24] http://www.aphis.usda.gov/brs/aphisdocs/03_10401p.pdf
[25] http://www.aphis.usda.gov/brs/aphisdocs/04_11001p.pdf

Deregulation of glyphosate-tolerant alfalfa likely would result in significant economic and environmental benefits from annual or semi-annual Roundup herbicide applications to glyphosate-tolerant alfalfa[26]. Forage quality is an important factor in the price paid for hay and the presence of weeds in the hay will reduce the quality considerably. For example, it is estimated that two applications of glyphosate at 0.75 lb a.i./acre would provide effective season-long control of troublesome weeds in California alfalfa fields. It is estimated that the Roundup herbicide cost would be $15/A, and a seed premium of $5/A would be charged. As mentioned previously, glyphosate is well recognized for the broad spectrum of weeds it controls. In fact, if a grower were to use a season-long herbicide control program that provides the same spectrum and performance as glyphosate, it would require the use of trifluralin, EPTC, and imazethapyr. The level of performance provided by these three herbicides would be full control of 38 species, partial control of eight species, and no control of two species found in California alfalfa seed production fields. This weed control program would require 4.7 lb. a.i./acre/yr. at a cost of $45/acre. Use of the glyphosate-tolerant alfalfa weed control program would provide full control of 42 species, partial control of 4 species, no control of one species with undetermined control of the remaining one species (University of California, 2001). Compared to the three herbicide program described above, the glyphosate weed control program would not only provide broader weed control but it would reduce herbicide inputs by 3.2 lb. a.i./acre and reduce costs by $25/acre.

The use of glyphosate-tolerant crops has resulted in a drastic reduction in the amount of total herbicide applied and a change in the herbicide active ingredient applied to U.S. cropland (Fernandez-Cornejo and Caswell, 2006). Deregulation of glyphosate-tolerant alfalfa and concomitant in-crop use of glyphosate is expected to reduce the total amount of herbicide use in alfalfa production. Therefore, the cumulative impact from deregulation of glyphosate-tolerant alfalfa is expected to be a further reduction in the amount of herbicide applied to U.S. cropland.

N.4.8 Other Analyses on Herbicide Use and Impacts from Commercial Use of Glyphosate-Tolerant Crops

Benbrook (2004) analyzed the USDA-NASS data from 1996 to 2004 and concluded that herbicide-tolerant crops have increased herbicide use by 138 million pounds with a 5% increase in herbicide use across the three major crops (corn, cotton, and soybean). Benbrook stated that the reliance on a single herbicide, glyphosate, as the primary method for managing weeds on millions of acres planted to herbicide-tolerant varieties remains the primary factor that has led to the need to apply more herbicides per acre to achieve the same level of weed control. These conclusions by Benbrook are not supported by the findings of Brooks and Barfoot (2006), Sankula (2006) or the USDA-ERS (2007).

USDA-ERS conducted a study entitled, "The First Decade of Genetically Engineered Crops in the United States" (Fernandez-Cornejo and Caswell, 2006). In 2001, 2002, and 2003 USDA-ERS surveys, growers responded that they adopted herbicide-tolerant crops for the following reasons: increased yields (60 – 67% of the responses), reduced pesticide input costs (11 – 17% of the responses), and decreased management time/ease of operation (15 – 26% of the

[26] USDA Petition 04-110-01p, Section D.6., p. 282, http://www.aphis.usda.gov/brs/aphisdocs/04_11001p.pdf

responses). This analysis found a cumulative reduction in herbicide use coinciding with the adoption of herbicide-tolerant crops. In addition, the report stated that glyphosate used on glyphosate-tolerant crops is less than one-third as toxic to humans and that glyphosate is not as likely to persist in the environment as the herbicides it replaces (Fernandez-Cornejo and McBride, 2002).

Based on the adoption rates of other glyphosate-tolerant crops, it is expected that glyphosate-tolerant alfalfa would replace a significant portion of conventional alfalfa varieties, especially where alfalfa is highly managed (e.g., Western and Southwestern states). As glyphosate-tolerant alfalfa is adopted, it is expected that Roundup herbicide will replace other forms of weed control currently used in alfalfa, as discussed below.

Glyphosate-tolerant alfalfa is highly tolerant to glyphosate and shows no yield loss or loss in forage quality when treated over multiple seasons over and above maximum glyphosate application rates (Pierson and Reyes, 2006). Recommended use rates are provided by Monsanto to growers in the Monsanto Technology Use Guide and Regional Technical Bulletins (See Appendix I of USDA petition 04-110-01p). In-season maximum application of glyphosate is up to 1.5 lbs a.e. per acre at a single application, and total per-year applications is 4.5 lbs a.e./acre. These application rates were used by Monsanto when conducting residue studies and for event selection purposes. In practice, these maximum rates will rarely be used since the vast majority of weeds are controlled using recommended rates and it would be uneconomical for growers to apply excess herbicide.

N.5 Impact on Soil Tillage Practices

Tillage is a farming practice used to prepare the seed bed and to mechanically remove weeds from the field. No-till farming, also known as conservation tillage or zero tillage, is defined as planting and growing crops without disturbing the soil through tillage. No-till and other conservation tillage farming practices improve soil and air quality, minimize surface runoff and soil erosion, enhance water quality, and reduce contributions to the greenhouse gases effect, particularly carbon dioxide (Fernandez-Cornejo and McBride, 2002; Leep et al., 2003). No-till seeding reduces equipment use, which can result in economic benefits of reduced labor and fuel cost. In the dryland areas of the northwest U.S., no-till seeding helps conserve soil moisture (G. Shewmaker, Extension Forage Specialist, University of Idaho, Personal Communications, 2007).

The main difference between no-till and conventional tillage seed practices is that no tillage is used to prepare the seedbed for seeding. When no-till seeding a field, a postemergence or burndown herbicide is applied prior to or at seeding to control any emerged weeds in place of tillage. Herbicide-tolerant crops facilitate the adoption of no-till farming; consequently, no-till farming has been extensively adopted for corn, cotton and soybean, for which herbicide-tolerant varieties are readily available. Data available for individual crops are discussed below.

N.5.1 Corn

As the adoption and commercial use of glyphosate-tolerant corn has increased, no-till corn acreage has steadily and significantly increased in parallel. In 2004 (the most recent year for which survey information is available), no-till practices were used on 19.7% of the total corn

acres, compared to zero no-till acres before glyphosate-tolerant corn was commercially available in 1996. The positive impacts from no-till production (such as reduced fuel use, soil erosion, runoff of pesticides and water, global warming potential, greenhouse gas emissions, and improved wildlife habitat) will increase as the adoption of herbicide-tolerant crops continues to increase (Leep et al, 2003; CTIC, 2004).

N.5.2 Cotton

The use of glyphosate-tolerant cotton has led to a significant increase in no-till production practices (Sankula, 2006). Total no-till cotton acres in 2004 were 2.4 million acres, or 18% of the total cotton acres. This is an increase of 371% compared to the no-till acres in 1996. The main reason for the increase in no-till cotton acreage since 1996 is the adoption of herbicide-tolerant cotton, allowing over-the-top glyphosate applications (Doane, 2002). Other reasons include enhanced grower awareness of the benefits of conservation tillage practices, increase in fuel prices, access to better no-till equipment, and availability of better herbicides to control weeds in no-till fields. Assuming that the entire no-till cotton acreage in 2004 (2.4 million acres) was planted to herbicide-tolerant varieties, fuel and labor cost savings were estimated to be $48 million (Sankula, 2006).

N.5.3 Soybean

In 1995, before the introduction of glyphosate-tolerant soybean, approximately 27% of the U.S. soybean acres used no-till production. By 2004, no-till acres increased to 36% of the total soybean acres (Sankula, 2006). A few states provide statistics on the adoption of no-till acres in their state. For 2007 in Indiana, no-till soybean was planted on 69% of the total soybean acres[27]. In 2006 in Illinois, no-till farming was used on 51% of the soybean acres. The University of Illinois Extension Service attributed that figure to the fact that 90% of the state's acres were planted to glyphosate-tolerant varieties, along with other factors, such as high fuel prices, improved equipment, higher yields using no-till practices, and better grower awareness of the advantages to soil and water quality from no-till farming[28].

N.5.4 Alfalfa

According to forage specialists, all alfalfa-growing regions currently practice some level of no-till seeding (J. Caddel, Forage Specialist, Oklahoma State University, Personal Communications, 2007; D. Hancock, Forage Specialist, University of Georgia, Personal Communications, 2007; D. Putnam, Extension Forage Specialist, University of California, Personal Communications, 2007; G. Shewmaker, Extension Forage Specialist, University of Idaho, Personal Communications, 2007; M. Sulc, Extension Forage Specialist, Ohio State University, Personal Communications, 2007; and D. Undersander, Extension Forage Specialist, University of Idaho, Personal Communications, 2007). All soil management groups (soil class

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[27] Summed from State of Indiana county level data from at http://www.in.gov/isda/files/Soybean_Rank_Percentage.pdf

[28] University of Illinois Extension Servive News Release: No-till is now the "Conventional" Tillage System for Illinois Farmers; http://web.extension.uiuc.edu/state/newsdetail.cfm?NewsID=4991

1 to 5) and all textures are suitable for no-till establishment and production provided the soils are naturally well-drained or tilled (Leep et al., 2003).

Few peer-reviewed publications are available to quantify the current acreage of no-till seeded alfalfa. Therefore, personal communication with local extension forage and weed specialists was heavily relied upon to address this question. Pennsylvania is the only state that conducts surveys quantifying no-till alfalfa acreage. The highest levels of no-till seeded alfalfa are practiced in the North Central, East Central, and Southern regions of the U.S. The amount of no-till varies significantly within these regions and states. No-till seeding in the North Central region can range from 5 to 20% of the seeded acres, with Minnesota practicing the lowest amount of no-till seeding (R. Becker, Weed Specialist, University of Minnesota, Personal Communications, 2007; S. Moeschnig, Extension Weed Specialist, South Dakota State University, Personal Communications, 2007; P. Murphy, NRCS Wisconsin, Personal Communications, 2007; D. Undersander, Extension Forage Specialist, University of Idaho, Personal Communications, 2007). Soil compaction plus cold and wet soil conditions in the spring are the primary reasons for the low adoption rate in Minnesota. The East Central region can range from 10 to 30% no-till[29] (Leep, 2003). Ohio reported the highest percentage of no-till. The no-till alfalfa acreage in the state of Pennsylvania is 21.4% based on actual survey results by the Pennsylvania NASS[30].

As with the other glyphosate-tolerant crops, the ability to use over-the-top treatments of glyphosate is expected to facilitate the further adoption of no-till practices. Deregulation of glyphosate-tolerant alfalfa, when considering the data from other glyphosate-tolerant crops, is expected to further increase the benefits from no-till farming. These benefits include improved soil and air quality, reduced surface runoff and soil erosion, enhanced water quality, and reduced contributions to the greenhouse gases effect, particularly carbon dioxide.

N.5.5 Other Crops

Limited information is available on the use of no-till farming practices in canola; however, a survey conducted in 2001 for the Canola Council of Canada (2001) did find that planting of herbicide-tolerant canola varieties resulted in an increase in the use of no-till practices. For sugar beet, more information should be available after more acres are planted in 2008; however, the presence of the herbicide-tolerant trait should allow for adoption of no-till practices, just as experienced for corn, cotton, and soybean.

N.6 Conclusions

Data show the introduction of glyphosate-tolerant crops has had no impact on the overland acres of crops planted in the U.S. The glyphosate-tolerant trait does increase grower flexibility in deciding what crop to plant, but has only a minor influence on the specific crop acreage planted, compared to other market forces, such as price. The planting of glyphosate-tolerant crops has resulted in a substantial decrease in the overall amount of herbicides applied to croplands. Use of glyphosate-tolerant crops has facilitated the adoption of no-till practices, which improve soil and water quality, reduce fuel use and reduce air pollution. Glyphosate-

[29] Sulc, M., Extension Forage Specialist, Ohio State University, Personal Communications, 2007.
[30] http://www.nass.usda.gov/Statistics_by_State/Pennsylvania/Publications/Survey_Results/tillage07_feb.pdf

tolerant crops increase grower flexibility regarding crop rotation but have minimal impacts on crop rotation.

According to USDA-ERS (2006), adoption of currently deregulated glyphosate-tolerant crops has resulted in an overall increase in farmer profitability, farmer household income, crop yield, soil conservation, and a reduction in herbicide use. Deregulation of other glyphosate-tolerant crops is expected to provide similar benefits to growers and the environment by reducing costs, reducing herbicide use, and increasing grower planting flexibility in crop choice.

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Appendix O. Potential Impact of Glyphosate on Human Health and the Environment

O.1 Overview

Glyphosate is a herbicide approved for use (registered) by the U.S. Environmental Protection Agency (EPA or the agency) for the control of weeds that would interfere with the growth of many food and non-food crops, including biotechnology-derived crops, as well as for control of weeds growing in non-crop areas. In 2001, EPA identified glyphosate as the most widely used conventional agricultural pesticide in the U.S. (Kiely et al., 2004). Glyphosate has been registered, and food and feed tolerances have been established in the U.S. for its residues, since 1979. Glyphosate has also successfully completed the reregistration process, as required for the continued registration of all pesticides originally registered before 1984.

Glyphosate has a complete and comprehensive regulatory data base (toxicity, environmental fate, and ecological toxicity) that has been evaluated by EPA to support all currently approved uses including glyphosate-tolerant alfalfa. EPA has stated that it has a high level of confidence in the quality of the existing studies and the reliability of the toxicity endpoints that are the basis for risk assessment (EPA, 2006a,b,c). In establishing food and feed tolerances to support the use of glyphosate on animal feed and forage crops (the group tolerance that supports the use of glyphosate in conventional and glyphosate-tolerant alfalfa), EPA noted that it had conducted "a complete and thorough review of the available data for glyphosate," and determined that "glyphosate will not pose unreasonable risks or adverse effects to humans or the environment" (EPA, 2002[31]).

The following discussion provides an overview of the regulatory and risk assessment processes applicable to glyphosate and all other agricultural use pesticides. Glyphosate has been approved by the EPA for a large number of food and feed uses, including uses associated with glyphosate-tolerant crops. Over 180 food and feed tolerances (40 CFR § 180.364) have been established for glyphosate in support of these uses. A complete listing of all U.S. glyphosate tolerances is provided in Attachment 1.

O.1.1 Pesticide Registration and Tolerance Setting

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires that before sale or distribution of a pesticide in the U.S., a person or company must obtain a registration, or license, from EPA. Before registering a new pesticide or a new use for a previously registered pesticide, EPA must first ensure that the pesticide, when used according to its label directions, will not cause unreasonable adverse effects on the environment. In order to address this standard, EPA must evaluate potential risks to humans and the environment, and may require applicants to submit more than 100 different scientific studies and tests conducted according to EPA guidelines. According to EPA, glyphosate is one of more than 1055 active ingredients currently registered as pesticides, which are formulated into many thousands of pesticide products that are available in the marketplace.

The process of registering a pesticide is a scientific, legal, and administrative procedure through which EPA examines the ingredients of the pesticide; the particular site or crop on

[31] Prior to 2002, separate alfalfa forage and alfalfa hay tolerances were established for glyphosate.

which it is to be used; the amount, frequency, method and timing of application, and other conditions of its use; and storage and disposal practices. In evaluating a pesticide registration application, EPA assesses a wide variety of potential human health and environmental effects associated with use of the product.

The data required by EPA are used to evaluate whether a pesticide has the potential to cause adverse effects on humans, wildlife, fish, and plants (including endangered species and NTOs, organisms that the pesticide is not intended to act against). The registration applicant must also supply data addressing the pesticide's potential impact on surface water or ground water (which might result from leaching or runoff, for example). Potential human health and safety risks range from short-term toxicity to long-term effects such as cancer and reproductive system disorders.

EPA also must approve the language that appears on each pesticide label. A pesticide product can only be used legally according to the directions for use on the labeling accompanying it at the time of sale. Following these directions carefully and precisely is necessary to ensure safe use.

A pesticide's registration is not the only opportunity EPA has to evaluate that product's safety. For example, EPA is currently completing a one-time program to review older pesticides (those initially registered before November 1984) under FIFRA to ensure that they meet current scientific and regulatory standards. This process, called reregistration, considers the human health and ecological effects of pesticides and results in actions to reduce risks that are of concern. EPA concluded its reregistration evaluation of glyphosate in 1993. At that time, the EPA produced a 291-page Reregistration Eligibility Decision document (RED) on glyphosate, setting forth the data on which it made a decision to reregister all then-existing uses of the pesticide, based on the pesticide having met the no unreasonable adverse effects standard found in FIFRA.

Where pesticides may be used on food or feed crops, EPA also sets tolerances (maximum pesticide residue levels) for the amount of the pesticide that can legally remain in or on foods. EPA undertakes this analysis under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). Under the FFDCA, EPA must find that such tolerances will be safe, meaning that there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue. This finding must be made and the appropriate tolerance established before a pesticide can be registered for use on the particular food or feed crop in question. Several factors must be addressed before a tolerance can be established, including:

- the aggregate, non-occupational exposure from the pesticide (exposure through diet, from using pesticides in and around the home, and from drinking water);
- the cumulative effects from exposure to different pesticides that produce similar effects in the human body;
- whether there is increased susceptibility to infants and children, or other sensitive subpopulations, from exposure to the pesticide; and
- whether the pesticide produces an effect in humans similar to an effect produced by a naturally occurring estrogen or produces other endocrine-disruption effects.

O.1.2 Pesticide Risk Assessment

The process EPA uses for evaluating the health impacts of a pesticide, under either FIFRA or the FFDCA, is called risk assessment. EPA uses the National Research Council's four-step process for human health risk assessment, which involves hazard identification, dose-response assessment, exposure assessment and risk characterization. Each of these steps is discussed below:

The first step in the risk assessment process is to identify potential health effects, or hazards that may occur from different types of pesticide exposure. EPA considers the full spectrum of a pesticide's potential health effects. Hazards are identified through a battery of studies that examine the potential toxicity of the pesticide in various tests including, where appropriate, tests with laboratory animals.

Generally, for human health risk assessments, many toxicity studies are conducted, based on EPA guidelines, by pesticide companies in independent laboratories following the Good Laboratory Practice (GLP) standards, and evaluated for acceptability by EPA scientists. EPA evaluates pesticides for a wide range of effects, from eye and skin irritation to cancer and birth defects. EPA may also consult the public literature or other sources of information on any aspect of the chemical.

The next step of the risk assessment considers the levels at which the pesticide produces adverse effects. Dose-response assessment involves considering the dose levels at which adverse effects were observed in test animals, and using these dose levels to calculate an equal dose in humans.

Step three of the process involves an exposure assessment. People can be exposed to pesticides in three ways:

1. Inhaling pesticides (inhalation exposure),
2. Absorbing pesticides through the skin (dermal exposure), and
3. Ingesting pesticides (oral exposure).

Depending on the situation, pesticides could enter the body by any one or all of these routes. Typical sources of pesticide exposure include agricultural (food); home and personal use pesticides; pesticides applied to lands that make their way into the drinking water; or occupational exposure for agricultural workers or pesticide applicators.

Risk characterization is the final step in assessing human health risks from pesticides. It is the process of combining the hazard, dose-response and exposure assessments to describe the overall risk from the use of a pesticide. It explains the assumptions used in assessing exposure as well as the uncertainties that are built into the dose-response assessment. The strength of the overall database is considered, and broad conclusions are made. EPA's role is to evaluate both toxicity and exposure and to determine the risk associated with use of the pesticide.

The risk to human health from pesticide exposure depends on both the toxicity of the pesticide and the likelihood of people coming into contact with it (exposure). At least *some* exposure and *some* toxicity are required to result in a risk. For example, if the pesticide is found to have a high level of toxicity, but people are not exposed to the pesticide, there is no risk. Likewise,

if there is ample exposure but the pesticide is nontoxic, there is no risk. However, usually when pesticides are used, there is some toxicity and exposure, which results in a potential risk.

EPA recognizes that effects vary between animals of different species and from person to person. To account for this variability, a 100-fold *uncertainty factor* is built into the risk assessment. This uncertainty factor creates an additional margin of safety for protecting people who may be exposed to the pesticides. FQPA requires EPA to use an extra 10-fold safety factor, if necessary, to protect infants and children from effects of the pesticide.

Once EPA completes the risk assessment process for a pesticide, the Agency uses this information to determine if (when used according to label directions), there is a reasonable certainty that the pesticide will not harm a person's health.

Using the conclusions of a risk assessment, EPA can then make a more informed decision regarding whether to approve a pesticide chemical or use, as proposed, or whether additional protective measures are necessary to limit occupational or non-occupational exposure to a pesticide. For example, EPA may prohibit a pesticide from being used on certain crops because consuming that commodity treated with the pesticide may result in an unacceptable risk to consumers. Another example of protective measures is requiring workers to wear personal protective equipment (PPE) such as a respirator or chemical resistant gloves, or not allowing workers to enter treated crop fields until a specific period of time has elapsed.

If, after considering all appropriate risk reduction measures, the pesticide still does not meet EPA's safety standard, the Agency will not allow the proposed chemical or use. Regardless of the specific measures enforced, EPA's primary goal is to ensure that legal uses of the pesticide are protective of human health, especially the health of children, and the environment.

O.2 Potential Impact of Glyphosate on Human Health

O.2.1 Glyphosate Safety Evaluations

Glyphosate presently has 186 established food and feed tolerances in the U.S (see Attachment 1). Each time EPA reviews an application to add a new food or feed use to the glyphosate label the Agency is required by FFDCA to conduct an aggregate risk assessment, considering all non-occupational sources of human exposure to the pesticide, and find that aggregate exposure to the pesticide will be safe as defined by the statute and regulations. Issues associated with potential occupational exposure for each new use are considered under FIFRA's unreasonable risk standard.

Over the course of these numerous reviews, the toxicology of glyphosate has been extensively studied. Comprehensive toxicological studies in animals have demonstrated that glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects or reproductive problems[32] (EPA, 1993; WHO/FAO, 2004). In fact, after a thorough review of all available

[32] European Commission (EC). 2002. Report for the Active Substance Glyphosate, Directive 6511/VI/99, January 21. http://europa.eu.int/comm/food/fs/ph_ps/pro/eva/existing/list1_en.htm.

toxicology data, the EPA concluded that glyphosate should be classified in Group E - Evidence of Non-carcinogenicity in Humans, the most favorable category possible (EPA, 1993).

Despite this extensive safety data, glyphosate safety is reviewed with every new use for which registration is sought, including, where necessary, uses associated with glyphosate-tolerant crops developed through biotechnology. As discussed above, prior to the approval of any new use of an existing registered pesticide, EPA must consider the potential human health effects from the aggregate (total combined) human exposure to that pesticide, combining the potential exposure from the proposed new use with all other existing exposures to the pesticide. Dietary exposure is considered, which addresses pesticide residues that may remain on food from crops on which the pesticide is applied (pre- or postemergence), as well as any residue that could be found in drinking water as a result of pesticide use. Non-dietary exposure is also included in this assessment, which includes exposure to the pesticide through residential use, such as on lawns or in flower beds, as well as exposure in a recreational context, such as from a golf course or sports field. Based on these data, EPA must be able to make a determination of reasonable certainty of no harm to human health as required by the FFDCA.

EPA does not conduct an acute dietary risk assessment for glyphosate because no acute effects have ever been identified in the toxicological studies conducted for glyphosate. Accordingly, EPA does not expect glyphosate to pose an acute risk (EPA, 2006d). EPA does conduct a chronic dietary (food and water) risk assessment for glyphosate based on a theoretical worst case exposure estimate. For food, this estimate assumes that glyphosate is used on 100 percent of all the crops on which the pesticide is currently approved for use. It further assumes that the resulting pesticide residues found on all harvested food crops are at the level of the legally established tolerance (i.e., the maximum allowable pesticide residue level). For water, EPA assumes that glyphosate is used to control weeds in water bodies by direct application to the water at the maximum application rate, without taking into account degradation in the water body (EPA, 2006b,c).

Applying this unrealistic, theoretical maximum exposure estimate, EPA determines how much of the established Reference Dose (RfD) would be utilized by all currently approved product uses. The RfD is an estimate of the amount of daily pesticide exposure to the human population that can occur over a lifetime with a reasonable certainty of no harm to human health.[33] For glyphosate, the RfD is 1.75 mg per kg body weight per day (mg/kg/day) (EPA, 2002). Provided that the utilization of the RfD from food and drinking water does not exceed the EPA level of concern of 100 percent [34, 35], EPA will then conduct an aggregate risk assessment (chronic and short/intermediate-term) to consider additional exposures from non-dietary exposure routes (residential and recreational).

If the aggregate risk assessment shows that utilization of the RfD does not exceed the EPA level of concern, then EPA will conclude that the new use does not pose an unreasonable risk to human health. EPA will then establish or revise, as needed, any food or animal feed crop

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[33] RfD is the current terminology used by EPA; however earlier EPA risk assessment terminology used the term Allowable Daily Intake (ADI). RfD and ADI are synonymous.

[34] EPA has reviewed state monitoring data on the occurrence of glyphosate in public drinking water systems and found that these data "reinforce the Agency's conclusion that aggregate exposure to glyphosate via all exposure routes, including drinking water, will not exceed the Agency's level of concern" (U.S. EPA, 2002).

[35] For glyphosate, the EPA's level of concern for chronic dietary exposure is 100 (U.S. EPA , 2006b).

tolerances to allow for the presence of glyphosate residue on that crop. EPA publishes these new tolerances in the Federal Register, along with a summary of the risk assessment and approves pesticide labeling for the new use. In issuing the final tolerance rule, EPA considers and discusses any comments received in response to the original notice regarding EPA's intention to establish tolerances that was published in the Federal Register.

Despite the large number of approved food and feed uses of glyphosate, including uses associated with glyphosate-tolerant crops, a large margin of safety exists for glyphosate. While use of glyphosate has increased in the decade since the introduction of glyphosate-tolerant crops, the associated risk to human health as a result of the increased human exposure to glyphosate remains low, due to the low mammalian toxicity of glyphosate and the relatively low dietary exposure associated with the herbicide's approved uses.

Prior to the first approval of a glyphosate-tolerant crop (soybean) in 1996, theoretical dietary exposure for all registered conventional uses of glyphosate utilized approximately 2.9% of the glyphosate RfD for the most sensitive subpopulation of non-nursing infants less than one year old (EPA, 1993). Incremental theoretical dietary exposure from the additional uses of glyphosate in glyphosate-tolerant soybean, cotton, corn, canola, alfalfa, sugar beet and creeping bentgrass combined with all other conventional crop uses of glyphosate approved between 1996 and 2006, account for only an additional 1% utilization of the glyphosate RfD for the subpopulation of infants less than one year old (EPA, 2004d). The total combined chronic aggregate exposure (dietary and non-dietary) from all uses of glyphosate utilizes well below 100% of the glyphosate RfD, only 9% for the most sensitive subpopulation of non-nursing infants less than one year old (EPA, 2006b). The total combined short/intermediate term aggregate exposure (dietary and non-dietary) for all current registered uses of glyphosate utilizes only 11% of the glyphosate RfD for the most sensitive subpopulation of non-nursing infants less than one year old (EPA, 2006b). The utilization of the glyphosate RfD, which is well below 100 percent, has allowed EPA to continue to make the conclusion of reasonable certainty of no harm to human health for each glyphosate use, including glyphosate-tolerant alfalfa and other new glyphosate-tolerant crop uses.

These figures are supported by the data provided in the tables below. Table O-1 summarizes the established food and feed tolerances supporting the use of glyphosate in the conventional crops of alfalfa, cotton, sugar beet and soybean prior to the first glyphosate-tolerant crop in 1996. A summary of the regulatory approvals, including new or modified food and feed tolerances, and associated dietary exposure assessments for approved glyphosate-tolerant crops is provided in Table O-2. Table O-3 summarizes the most recent chronic and short/intermediate-term aggregate risk assessments for glyphosate.

Table O-1. Established Glyphosate Tolerances Prior to Glyphosate-tolerant Crops (1993)

Crop	Established Food/Feed Tolerances	Publication	% of Reference Dose (RfD)
Soybean	• Seed – 20 ppm • forage & hay – 15 ppm • hulls – 100 ppm	Glyphosate Reregistration Eligibility Decision Document September 1993 (EPA, 1993)	General Population - 1.2 Non-nursing infants <1 year old - 2.9
Alfalfa	200 ppm		
Cotton	forage, hay, & seed – 15 ppm		
Sugar beet	Roots – 0.2 ppm		

Table O-2 (continued). Summary of EPA Approvals for Glyphosate Use in Glyphosate-tolerant Crops

	Commercial Introduction Year	Required Changes in Food/Feed Tolerances	Federal Register Publication Establishing New or Modified Tolerance	% of Reference Dose (RfD) Dietary Exposure Only (Food + Water)
Roundup Ready soybean	1996	• Increase soybean forage to 100 ppm. • Increase soybean hay to 200 ppm. • Establish new tolerance for aspirated grain fractions at 50 ppm.	61 FR 15192 Petition No. 4F4369 Apr. 1996 (EPA, 1996b)	General Population – 1 Non-nursing infants- 2.5
Roundup Ready cotton	1997	Establish new tolerance for gin byproduct at 100 ppm.	61 FR 7729 Petition No. 5F4493 Feb. 1996 (EPA, 1996a)	General Population - 1 Non-nursing infants - 2.4
Roundup Ready corn	1998	Establish new tolerance for corn forage at 1 ppm.	62 FR 17723 Petition No. 5F4555 Apr. 1997 (EPA, 1997)	General Population - 1 Non-nursing infants < 1 year old - 3
Roundup Ready canola	1999	Establish new tolerances for canola. • seed at 10 ppm • meal at 15 ppm	64 FR 18360 Petition No. 2E4118 Apr. 1999 (EPA, 1999)	General Population - 1.5 Non-nursing infants <1 year old - 3.3
Roundup Ready sugar beet	2008	Establish new tolerances for sugar beet. • roots at 10 ppm • tops at 10 ppm • pulp (dried) at 25 ppm		
Roundup Ready corn 2	2004	Increased tolerance for corn forage to 6 ppm.	68 FR 36472 Jun. 2003 (EPA, 2003)	Change in forage tolerance did not affect estimated dietary exposure from animal products; therefore no dietary risk assessment was conducted.
Roundup Ready Flex cotton	2006	• Increase tolerance for gin byproduct to 175 ppm. • Increase tolerance for cottonseed to 35 ppm.	69 FR 65081 Petition No. 3F6570 Nov. 2004 (EPA, 2004d)	General Population - 2.2 All infants < 1 year old - -3.9
Roundup Ready alfalfa	2006	Establish new tolerances for alfalfa seed at 0.5 ppm. Existing tolerances for alfalfa forage (400 ppm) and hay (200 ppm) were sufficient to cover new in-crop uses.	70 FR 7861 Petition No. 2F6487 Feb. 2005 (EPA, 2005)[1]	Dietary exposure insignificant, did not conduct new risk assessment. Deferred to assessment conducted for flex cotton as published in 69 FR 65081.

[1]U.S. Environmental Protection Agency. 2005. Final Rule. Federal Register Vol. 70, No. 31: 7861. Feb. 2005.

Table O-3. Aggregate Exposure Assessment for Glyphosate

Population Subgroup	Acute Aggregate[2]	RfD (mg/kg/day)[2]	Chronic Aggregate[1,2]		Short/Intermediate Term Aggregate[2]	
			Exposure (mg/kg/day)	% RfD	Exposure (mg/kg/day)	% RfD
General U.S. population	Not applicable	1.75	0.041	2	-	-
All infants (<1 year)			0.127	7	0.157	9
Non-nursing infants (<1 year)			**0.158**	**9**	**0.188**	**11**
Children 1-2 years			0.095	5	0.125	7
Children 3-5 years			0.088	5	0.118	7
Children 6-12 years			0.059	3	0.089	5
Youth 13-19 years			0.037	2	-	-
Adults 20-49 years			0.033	2	0.063	4
Adults 50+ years			0.028	2	-	-
Females 13-49 years			0.031	2	-	-

[1]Chronic aggregate exposure is the same as chronic dietary exposure because chronic non-dietary exposure is not expected based upon the current registered non-crop uses of glyphosate.

[2]EPA OPPTS. Glyphosate Human Health Risk Assessment for Proposed Uses on Safflower and Sunflower. Petition No. 4E6878. Sept. 5, 2006.

O.2.2 Glyphosate Safety Evaluation for Applicator and Bystander Exposure

Another potential impact of the use of glyphosate on human health that EPA considers in its human health analysis is applicator and bystander exposure resulting from increased glyphosate use. Based on the toxicity of glyphosate and its registered uses, including use on glyphosate-tolerant crops, EPA has concluded that occupational exposures (short-term dermal and inhalation) to glyphosate are not of concern because no short-term dermal or inhalation toxicity endpoints have been identified for glyphosate (EPA, 2006b; EPA, 2006c).

Additional evidence to support the EPA conclusion can be found in the Farm Family Exposure Study (Acquavella et al., 2004), a biomonitoring study of pesticide applicators conducted by independent investigators. This biomonitoring study determined that the highest estimated bodily adsorption of glyphosate as the result of routine labeled applications of registered glyphosate-based agricultural herbicides to crops, including glyphosate-tolerant crops, was approximately 400 times lower than the RfD established for glyphosate. Furthermore, investigators determined that 40% of applicators did not have detectable exposure on the day of application, and 54% of the applicators had an

estimated bodily adsorption of glyphosate more than 1000 times lower than the RfD (Acquavella et al., 2004).

The biomonitoring study also found little evidence of detectable exposure to individuals on the farm who were not actively involved in or located in the immediate vicinity of labeled applications of glyphosate-based agricultural herbicides to crops. Considering the similarity of the use pattern and application rates of the glyphosate products in this study compared to those registered for use on glyphosate-tolerant alfalfa and glyphosate-tolerant crops in general, bystander exposure attributed to the use of glyphosate on glyphosate-tolerant crops is expected to be negligible.

O.3 Potential Impact of Glyphosate on the Environment

Potential environmental effects are carefully considered as a part of the FIFRA pesticide registration process. Prior to the approval of a new pesticide or a new use (including a change in pesticide application rates and/or timing) and before reregistering an existing pesticide, EPA must consider the potential for environmental effects and make a determination that no unreasonable adverse effects to the environment will be caused by the new pesticide, new use or continued use.

To make this determination, EPA requires a comprehensive set of environmental fate and ecotoxicology data on the pesticide's active ingredient (40 CFR Part 158). EPA uses these data to assess the pesticide's potential environmental risk (hazard x exposure). The required data include both short and long-term hazard data on representative organisms that are used to predict hazards to terrestrial animals (birds, nontarget insects, and small mammals), aquatic animals (freshwater fish and invertebrates, estuarine and marine organisms), and nontarget plants (terrestrial and aquatic).

EPA reevaluated the environmental safety of glyphosate in 1993 as part of the FIFRA-required reregistration of all pesticides. At the end of this evaluation, EPA concluded that all registered uses of glyphosate were eligible for reregistration, including terrestrial (i.e., land-based) applications up to 7.5 pounds glyphosate acid equivalents (a.e.) per acre.

Since the reregistration evaluation in 1993, EPA has reviewed and approved a significant number of new glyphosate uses: conventional crops such as legume vegetables and sunflower/safflower seed, glyphosate-tolerant crops such as corn, cotton, canola and soybean, and non-crop areas. In each case, EPA concluded that the new use, including any incremental environmental exposure to glyphosate caused by that new use, did not pose an unreasonable risk to the environment, and approved pesticide labeling for the new use.

The studies and data collected by Monsanto, both for the initial EPA registration and reregistration of glyphosate, as well as data developed by independent academics, present a well-established safety profile for glyphosate. The following sections provide greater detail regarding some of the key findings from these studies.

O.3.1 Persistence of Glyphosate in the Soil

Persistence of agricultural chemicals in the soil is widely regarded as an undesirable environmental characteristic. Glyphosate has been shown to rapidly dissipate from most agricultural ecosystems across a wide range of soil and climatic conditions, with a median soil half-life (the time it takes for half of the glyphosate to dissipate in the soil) of 13 days (Giesy et al., 2000). The potential for glyphosate to accumulate in soil following repeated applications has been studied both in the laboratory and the field.

A laboratory study was conducted on two soil samples, with each sample receiving up to three sequential applications of 5 pounds glyphosate a.e. per acre over a 6-week period, at two-week intervals. The concentration of glyphosate in soil 24 weeks following application had declined to 1-5% of the concentration immediately after application, regardless of whether it was the first, second or third application.

Glyphosate degradation in the soil following multiple glyphosate applications was also shown under field conditions. Soil was collected from pesticide efficacy and tolerance trials in orchards and vineyards that received repeated applications of glyphosate over a one- to six-year period, at cumulative rates of 6 to 120 pounds glyphosate a.e. per acre. These soil samples did not show any accumulation of glyphosate residues, even at the exaggerated rate of three sequential applications of eight pounds glyphosate a.e. per acre within a three-month interval for five out of six sequential years. Glyphosate degradation continued after multiple applications, and less than 10 percent of the total applied glyphosate remained in the soil one year after the last glyphosate application.

A typical agronomic (annual) use pattern for glyphosate on glyphosate-tolerant crops is a preemergence burn down application of 1.5 pounds glyphosate acid per acre followed by one to two postemergence applications ranging from 0.77 to of 1.5 pounds glyphosate a.e. per acre, and where sequential application intervals are longer than two to four weeks. The maximum labeled rates and typical use patterns of glyphosate on glyphosate-tolerant crops are well within the rates and frequencies used in the studies above. As a result, glyphosate is not expected to accumulate in soil as a result of labeled uses in glyphosate-tolerant crop.

O.3.2 Persistence of Surfactant in the Soil

Pesticide products approved for application to emerged weeds normally are applied with surfactants. Glyphosate products are formulated with surfactants to increase the permeability of the cuticle wax of the weed foliage to increase the foliar uptake of glyphosate. In other words, the surfactant acts to break down the plant's natural protective wax coating, allowing the plant to better absorb the glyphosate, thereby improving the efficacy of the herbicide.

The predominant type of surfactant used in formulated glyphosate products worldwide is polyethoxylated alkyl amine (POEA). When degradation of POEA was investigated in three types of soil (silt loam, silty clay loam, and sandy loam), microbial degradation was determined to be the primary degradation route, with minimal degradation occurring under sterile conditions. Approximately 25-30% of applied ^{14}C-POEA was mineralized to $^{14}CO_2$ within seven weeks. The estimated degradation half-life for parent POEA was less

than one week and possibly as short as one to two days. Because limited data are available for POEA dissipation, a conservative estimate of half-life values for POEA in soil would be 7-14 days[36] (Giesy et al., 2000). Glyphosate and the POEA surfactant have similar soil dissipation rates and the same primary route of dissipation, i.e., microbial degradation. Therefore, it is reasonable to assume that the POEA surfactant will behave similarly to glyphosate in field soil, and an increase in residual soil concentrations (accumulation) of the POEA surfactant is not anticipated as a result of increased use of glyphosate associated with the planting of glyphosate-tolerant crops in general.

O.3.3 Surface Water and Groundwater

Glyphosate binds strongly to soil and has a low potential to move offsite to surface water or leach to groundwater (EPA, 1993). The EPA has estimated glyphosate levels that could occur in surface water based on presently approved use patterns. Relying on toxicological data from acute and chronic tests on fish and other aquatic organisms, EPA has determined that "the potential for environmental effects of glyphosate in surface water is minimal" (EPA, 2002).

O.3.4 Wildlife

1. Animals: As a part of the reregistration evaluation under FIFRA, EPA conducted an ecological assessment for glyphosate. This assessment compared the results from toxicity tests with glyphosate conducted with various plant and animal species to a conservative estimate of the concentration of glyphosate to which an organism might be exposed in the environment. This estimate, called the Estimated Exposure Concentration (EEC), is a point estimate for exposure that does not take into account normal environmental dilution or dissipation, or the frequency of exposure to the pesticide by wildlife. In the Reregistration Eligibility Decision (RED) for glyphosate (EPA, 1993), the exposure estimates were determined assuming an application rate of 5.0625 lb a.e., which exceeds the maximum labeled use rate for agricultural purposes. When the EECs were calculated for aquatic plants and animals, the direct application of this rate to water was assumed. Based on this assessment, EPA concluded that effects to birds, mammals, fish and invertebrates are minimal based on available data (EPA, 1993).

Glyphosate is practically nontoxic to honey bees (which are used to assess effects on nontarget insects in general) and practically nontoxic to slightly toxic to birds, freshwater fish, marine and estuarine species, aquatic invertebrates and mammals (EPA, 1993). Glyphosate has a low octanol-water coefficient, indicating that it has a tendency to remain in the water phase rather than move from the water phase into fatty substances; therefore, it is not expected to accumulate in fish or other animal tissues.

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[36] Marvel, J.T., Brightwell, B.B. and Suba, L.A. 1974. G3780A Surfactant: Biodegradation, plant uptake and ^{14}C Distribution. Monsanto Company Report No. 321

The glyphosate end-use products used in agriculture contain a surfactant to facilitate the uptake of glyphosate into the plant (Ashton and Crafts, 1981). Depending on the surfactant used, the toxicity of the end-use product may range from practically nontoxic to moderately toxic to fish and aquatic invertebrates (EPA, 1993). For this reason, the 1993 Glyphosate RED stated that some formulated end-use products of glyphosate needed to be labeled as "Toxic to fish" if they were labeled for direct application to water bodies. Due to the associated hazard to fish and other aquatic organisms, glyphosate end-use products that are labeled for applications to water bodies generally do not contain surfactant.

2. Plants: Glyphosate is a non-selective herbicide with activity on essentially all annual and perennial plants. As such, exposure to glyphosate could put aquatic and terrestrial nontarget plants as well as threatened and endangered plants at risk (EPA, 1993). Nontarget plants may potentially be at risk from applications of glyphosate as a result of spray drift. As discussed earlier, glyphosate binds tightly to soil and does not move offsite. Moreover, glyphosate is not taken up from agricultural soils by plants. Therefore risks to nontarget plants are only attributed to the spray drift of the pesticide. Pesticide labels include specific risk management measures to manage spray drift, including mandatory requirements for aerial applications.

During the reregistration process in 1993, additional data on terrestrial nontarget plants were requested by the EPA. These additional data have been utilized in conjunction with an exposure assessment to further understand the potential risk to threatened and endangered plants from the use of glyphosate[37] (Mortensen et al., 2008*)*.

O.3.5 Endangered and Threatened Species

The EPA Endangered Species Protection Program web site, http://www.epa.gov/espp/, describes the EPA assessment process for endangered species. The essential elements of that process, generally taken from the web site, are summarized below.

The Endangered Species Act (ESA) was intended to protect and promote the recovery of animals and plants that are in danger of becoming extinct. All federal agencies are required under the ESA to ensure that their regulatory actions, including EPA's registration of pesticides in the U.S., are not likely to jeopardize the continued existence of threatened or endangered species ("listed" species) or destroy or adversely modify their critical habitat.

EPA's Endangered Species Protection Program (ESPP) helps promote the recovery of listed species. The ESPP is a program designed to determine whether pesticide use in a certain geographic area may affect any listed species.

When registering a pesticide or reassessing the potential ecological risks from use of a currently registered pesticide, EPA evaluates extensive toxicity and ecological effects

[37] Mortensen, SR, Carr, KH, and Honegger, JL. 2008. Tier I Endangered Species Assessment for Agricultural Uses of Glyphosate and Glyphosate-Containing Herbicides. Monsanto Study Number RPN-2007-227. January 2008.

data to determine how a pesticide will move through and break down in the environment. Risks to birds, fish, invertebrates, mammals and plants are routinely assessed and used in EPA's determinations of whether a pesticide may be licensed for use in the U.S.

EPA's core pesticide risk assessment and regulatory processes ensure that protections are in place for all populations of nontarget species. Because endangered species may need specific protection, EPA has developed risk assessment procedures described in the Overview of the Ecological Risk Assessment Process (EPA, 2004a) to determine whether individuals of a listed species have the potential to be harmed by a pesticide, and if so, what specific protections may be appropriate. EPA's conclusion regarding the potential risks a pesticide may pose to a listed species and any designated critical habitat for the species, after conducting a thorough ecological risk assessment, results in an "effects determination."

An assessment of the effects of glyphosate use on all types of threatened and endangered species was conducted by Monsanto. This assessment generally followed the procedures described in the Overview of the Ecological Risk Assessment Process (EPA, 2004a), as summarized in Figure O-1.

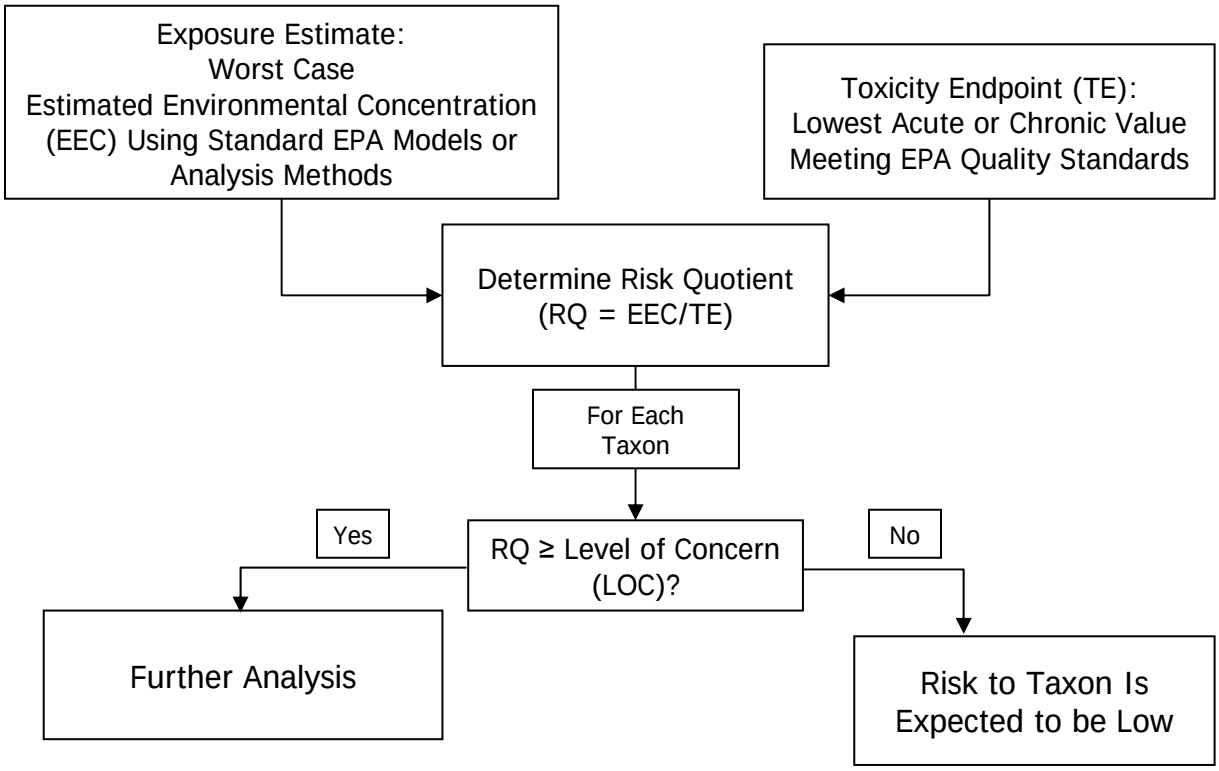

Figure O-1. Tier I Endangered Species Assessment

Risk quotients (RQ's) were calculated as the quotient of the EEC and the relevant toxicity endpoint for the most sensitive species for a given taxon (class of species). For acute studies of a few days duration, the concentration calculated to result in 50% mortality (LC_{50}) or 50% designated effect (EC_{50}) on the test species was utilized in the RQ calculation. For chronic studies, representing a significant portion of the species life-cycle, the highest concentration at which no effects were observed (No Observed Effect Concentration, NOEC) was used in the RQ calculation.

Toxicity values (effects endpoints) for most categories of species were taken from the EPA assessment for new glyphosate uses on bentgrass (EPA, 2006a), or from EPA guideline studies conducted by Monsanto if these endpoints were lower. Studies from the literature were considered when the study design was appropriate for the assessment being made and where sufficient information regarding glyphosate or formulation test concentrations was available. Exposure estimates were based on standard EPA methods for calculating exposure (EPA, 2004a). For aquatic organisms, the model GENEEC2 (EPA, 2004a), which calculates high-end estimates of surface water concentrations of pesticides in a generic farm pond, was utilized. When formulation toxicity was

considered, default drift values and the EPA standard pond[38] were utilized for estimation of aquatic exposure. For terrestrial animals, the T-Rex model[39] was utilized to calculate estimated dietary exposure and risk. For terrestrial and semi-aquatic plants, only the drift component of the TerrPlant model (EPA, 2004a) was used to determine exposure levels (the runoff component was disregarded). Runoff was not considered to contribute to exposure, since glyphosate binds very tightly to agricultural soils and does not have herbicidal properties when bound to soil (EPA, 2006a).

The conclusion from this assessment, submitted to USDA is that threatened or endangered terrestrial or semi-aquatic plant species are not at risk from ground applications of glyphosate at rates less than 3.5 lb glyphosate (a.e.) per acre, or from aerial applications at rates less than 0.70 lb a.e. per acre. However, these species may be at risk when rates exceed these levels. Since the maximum single application rate before or after crop emergence in glyphosate-tolerant alfalfa is 1.55 lb a.e. per acre, no listed plant species are predicted to be at risk from ground application of glyphosate to glyphosate-tolerant alfalfa. For other glyphosate-tolerant crops, in-crop application rates are typically less than 3.5 lb a.e. per acre, resulting in a prediction of no risk to listed plant species. Rates that exceed 3.5 lb a.e. per acre are generally for control of perennial species prior to crop emergence or prior to harvest.

The same assessment determined that other taxa (including birds, mammals, insects, fish, amphibians, aquatic invertebrates, and aquatic plants) were not at risk from the use of glyphosate herbicides in alfalfa production or in the production of other crops. Furthermore, this assessment determined that these other taxa were not at risk from indirect effects resulting from habitat alteration from the use of glyphosate, since non-endangered terrestrial or semi-aquatic plants were not considered to be at risk of direct effects.

Based on Monsanto's determination that threatened and endangered plant species may be at risk from certain uses of glyphosate in crop production (e.g., aerial application), a more detailed evaluation of the locations of threatened and endangered plant species relative to areas of crop production has been undertaken. The first crop to be assessed was alfalfa. The assessment process was divided into three phases, as outlined below.

- First, the co-occurrence of observations of threatened and endangered plant species and the presence of alfalfa production was determined at the county level[40,41]. (Phase 1) This assessment considered the 2663 counties in which

[38] A water body with a depth of 2 m and a volume of 20,000 liters.

[39] U.S. Environmental Protection Agency. 2006. T-REX (Terrestrial Residue EXposure Model). Version 1.3.1.

[40] Priester T, Kemman R, Rives Frank A, Turner L, McGaughey B, Howes D, Giddings J, Dressel S. 2007. An Analysis of Possible Risk to Threatened and Endangered Plant Species Associated with Glyphosate Use in Alfalfa: A County-Level Analysis. Monsanto Study Number CS-2005-125.

[41] Priester T, Kemman R, Rives Frank A, Turner L, McGaughey B, Howes D, Giddings J, Dressel S. 2008. An Analysis of Possible Risk to Threatened and Endangered Plant Species Associated with Glyphosate Use in Alfalfa: A County-Level Analysis (Supplement). Monsanto Study Number CS-2007-229.

alfalfa is grown, which comprise 85% of the 3141 counties and equivalent areas[42] in the 50 states of the U.S.

- Next, in counties with both threatened or endangered plant species observations and alfalfa production, the possible exposure of threatened and endangered plant species to glyphosate was assessed at the sub-county level. (Phase 2) This assessment used information available at the sub-county level for threatened and endangered plant species locations and for land use. Land uses considered in this assessment are identified as Pasture/Hay and Cultivated Crops.[43]
- Finally, in subcounty areas where, under certain application conditions, the potential for threatened and endangered plant species to be at risk from exposure to glyphosate could not be excluded, these areas have been defined so that grower practices can be implemented to limit glyphosate exposure. Measures to limit glyphosate exposure in these areas will be proposed. (Phase 3) These measures include (1) limiting ground application rates to less than 3.5 lb glyphosate a.e. per acre in areas identified for potential use limitation when the potential habitat for the threatened or endangered species is present, and (2) for aerial applications implementing an unsprayed buffer between the potential habitat for the listed species and the application area. Buffers are proposed to be based on application rate, droplet size and wind direction.

This analysis has been completed for the 2663 U.S. counties in which alfalfa is grown. The evaluation of the remaining 478 counties of the U.S. is currently in progress, based on the presence of threatened or endangered plant species, the production of other crops in those counties, and the cultivated crop or pasture/hay land use. In counties that have observations of threatened and endangered plant species and agricultural land uses, a more detailed sub-county analysis will be conducted.

Of the 2663 U.S. counties where alfalfa is produced, less than 11% of counties (284 counties) have required the definition of potential areas for use limitations. In the other 2379 counties, either there are no threatened or endangered plant species present, or the species present are either excluded from concern (based on habitat or proximity information), have existing protections, or are not in proximity to potential areas of alfalfa production that are not already excluded or protected in some way.

The Roundup Ready alfalfa assessment considered all land that could potentially be used for agricultural crop production (in counties with reported alfalfa farms) in the assessment of proximity to observations of threatened or endangered species. Thus, the identification of potential use limitation areas also applies to other crops in those counties. Because alfalfa is grown in such a large number of counties, the endangered plant species assessment conducted for alfalfa covers more than 90% of the acres where corn, cotton, soybean, sugar beets and canola are grown.

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[42] Equivalent areas include independent cities that are not within the boundaries of a county.

[43] Land use was based on the National land Cover Database (2001) for the continental U.S. and on the NOAA (National Oceanic and Atmospheric Administration) Coastal Services Center land cover data for Hawaii.

O.3.6 Potential Effects on Endangered Animal Species Identified by EPA or in Litigation

As previously discussed, no indirect effects on threatened or endangered animal species are predicted, since no significant direct effects due to pesticide drift onto non-endangered plant species are predicted. In the Glyphosate Reregistration Eligibility Decision (RED) (EPA, 1993), EPA suggested that glyphosate may have effects on the habitat of the Houston Toad. After the issuance of the 1993 Glyphosate RED, Monsanto conducted a vegetative vigor study. When relevant effects data from that study are considered, it can be determined that the amount of glyphosate per unit area predicted to drift away from the site of an agricultural application is less than the amount per unit area observed to have a 25% effect on plant dry weight or growth of the most sensitive of ten species tested in the study. Thus, the habitat of the toad is not likely to be significantly affected by glyphosate drift, and hence the toad is not likely to be at risk from the agricultural use of glyphosate. Similar conclusions can be drawn with respect to the habitat of other endangered animal species, such as the California red-legged frog (which has been the subject of years-long litigation between public interest groups and the federal government[44]). The assessment conducted also indicates that the frog itself is not at risk from aquatic exposures to glyphosate used in agriculture because estimated exposure concentrations are much lower than concentrations at which effects on amphibians have been observed.

The EPA also has evaluated the potential effect of glyphosate on salmon in eleven areas in California and Southern Oregon[45] in response to the consent agreement reached in another lawsuit[46]). The conclusion of the EPA's risk assessment is as follows:

"For all uses with application rates of 5 lb a.i. per A or below, the Agency has determined that glyphosate will have No Effect on the subject listed species." (EPA, 2004b,c). All glyphosate use rates for agricultural uses are 5 lb a.i. per acre (3.75 lb glyphosate a.e. per acre) or below, so no risk to salmon is anticipated from these uses.

O.3.7 Other Potential Environmental Impacts Associated with Glyphosate Use in Glyphosate-tolerant Crops

As discussed more fully below, the potential impacts to soil attributable to the change in production (cultivation) practices associated with the deregulation of glyphosate-tolerant crops have been assessed. The adoption of glyphosate-tolerant crops and the ability to use glyphosate-based agricultural herbicides is not expected to significantly change agricultural practices, except to enable the adoption of no-till seeding practices.

1. No-Till Practices: No-till production is the practice of establishing an agricultural seed bed and controlling weeds without mechanically tilling the soil. Instead, the only tillage of the soil is done at the time of planting, with the crop being seeded directly into the previous year's crop residue. Among other environmental benefits, no-till production

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[44] *Center For Biological Diversity v. Leavitt*, 2005 WL 2277030 (N.D.Cal., September 19, 2005).
[45] These areas are call Evolutionarily Significant Units based on the salmonid populations present in these areas.
[46] , *Washington Toxics Coalition v. Environmental Protection Agency*, 413 F.3d 1024 (9th Cir. 2005).

reduces soil erosion and the use of petroleum-based fuels for tractors. The practice has been shown to minimize surface water runoff and soil erosion and to improve soil quality by increasing the soil organic matter that helps bind soil nutrients and prevent their loss to runoff, erosion and leaching (Leep et al., 2003). Less soil erosion into surface waters would positively impact stream dynamics (McVay et al., 2005).

No-till agriculture can provide benefits to water bodies, as well. No-till practices reduce soil erosion to surface water bodies, decreasing the amount of sediment in rivers and streams. Sedimentation increases the turbidity (cloudiness) of surface water bodies, reducing light penetration, impairing photosynthesis and altering oxygen levels, which cause a reduction of food sources for some aquatic organisms. Sediment can also cover spawning beds and impact fish populations. Phosphorus (a major component of fertilizer) bound to soil particles can be transferred to rivers and lakes via soil erosion, giving rise to high levels of phosphorus in surface waters, which may lead to algae blooms that can impact desirable fish populations (Hill et al., 1995).

2. Soil Microorganisms: Soybean is a legume that forms a symbiotic relationship with the nitrogen-fixing bacterium *Bradyrhizobiacea japonicum* (see Section VIII.D.4). The effects of glyphosate and glyphosate-based formulations on soil microorganisms have been extensively investigated (Sullivan and Sullivan, 2000). Results of standardized tests with glyphosate formulations performed for submission to regulatory agencies indicate no long-term effects on microorganisms in soil even at rates that exceed maximum use rates (up to five times the labeled rate). In addition, independent researchers have reviewed numerous laboratory and field studies, investigating the effects of glyphosate on soil bacteria and fungi[47] (Giesy et al., 2000). Although some laboratory tests have shown effects on nitrogen-fixing bacteria (Moorman et al., 1992; Santos and Flores, 1995) and soil fungi (Estok et al., 1989; Busse et al., 2001), effects are typically observed only under artificial laboratory conditions and at glyphosate concentrations well above normal field application rates. Several researchers have concluded that it is difficult to extrapolate results from the laboratory to the natural soil environment (Estok et al., 1989; Wan et al., 1998; Busse et al., 2001).

In studying microorganisms from soil in pine plantations, Busse et al. (2001) state: "Our findings suggest that artificial media assays are of limited relevance in predicting glyphosate toxicity to soil organisms and that field rate applications of glyphosate should have little or no affect on soil microbial communities in ponderosa pine plantations." Long-term studies following repeated applications of Roundup agricultural herbicides in the field for six (Olson and Lindwall, 1991) or over 10 years (Hart and Brookes, 1996; Biederbeck et al., 1997) have shown no detectable adverse effects on soil microbes. Investigations by Haney et al. (2000, 2002) related to the increased use of glyphosate-tolerant crops indicate that glyphosate was degraded over time by soil microbes, even at high application rates, without adversely impacting the soil microbial community. In

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[47] Felsot, A.S. 2001. Herbicide tolerant genes, Part 4: Withering wildlife? Agric. & Environ News, No. 178. http://www.aenews.wsu.edu/Feb01AENews/Feb01AENews.htm.

addition, results from field studies that have evaluated the fungal component of the soil microbial community indicate that glyphosate treatment had no deleterious effects on beneficial soil fungi (Araujo et al., 2003; Biederbeck et al., 1997; Busse et al., 2001; Wardle and Parkinson, 1990a,b). Moreover, the history of safe use and yield data obtained for nearly 10 years of glyphosate-tolerant crop production, combined with in-crop applications of glyphosate-based agricultural herbicides, reinforce the findings that soil microbes and microbially mediated processes are not adversely impacted by field-rate applications of glyphosate.

3. The Potential for Glyphosate Metal Chelation to Affect Soil Fertility: Plants are dependent on the uptake of a number of different metal cations from the soil for optimal growth. Glyphosate is known to chelate, or tightly bind, to several di- and trivalent metal cations such as Fe3+, Cu2+, Mn2+, Al3+, and Ca2+ that are needed by plants (Madsen et al., 1978; Glass, 1984). Cations that chelate glyphosate have been shown to reduce the efficacy of glyphosate when present in sufficient amounts in the tank mix spray solution[48]. In the spray solution, there is a simple interaction between glyphosate and metal cations, which reduces the herbicidal activity of glyphosate. However, in the soil environment, the interactions between metals and chelators are much more complex (Parker et al., 2005). Glyphosate can interact with metals that are present on the surface of soil particles, as well as with dissolved metal ions in the water soil solution. In addition to glyphosate, many other potential ligands or chelators are present in soil that can also interact with metals. As a result, there is a complex multi-component equilibrium between glyphosate, other ligands or chelators, and numerous metals present in soil. Glyphosate is only one factor in this system. Numerous compositional analysis studies have demonstrated a lack of any significant immobilization of mineral nutrients by glyphosate in soil that results in reduced uptake by plants. These studies have shown that glyphosate-tolerant crops that have been sprayed with glyphosate do not have decreased micronutrient levels compared to untreated controls (McCann et al., 2006; Obert et al., 2004; Ridley et al., 2002).

4. Nitrogen Fixation: Nitrogen fixation is the process by which inorganic nitrogen is fixed into an organic form necessary for plant growth. It occurs in root nodules that are an integral part of the legume root system. The process of nitrogen fixation is subject to subtle variations depending on soil type and environmental conditions. While some laboratory investigations have indicated that glyphosate may inhibit pure cultures of nitrogen-fixing bacteria (Moorman et al.1992; Santos and Flores, 1995), effects were only observed at glyphosate concentrations above normal field application rates.

Several researchers (King et al., 2001; Hoagland et al., 1999; Goos et al., 2002; Zablotowicz and Reddy, 2004) have investigated potential effects of glyphosate formulated herbicides on nitrogen-fixing bacteria associated with glyphosate-tolerant

[48] de Ruiter, H., R.A. Downer, A.J.M. Uffing, T.A. Ebert, P.J.J. Pikaar and F.R. Hall. 2001. The influence of inorganic cations on glyphosate activity - review and perspectives. American Society for Testing and Materials, West Conshohocken.

soybean. In general, any effects observed on the formation of nodules or nitrogen fixation were not observed uniformly and were noted to be transient in nature. Hoagland et al. (1999) reported some reduction in nodulation in Roundup Ready soybean, but noted that effects were of minimal consequence due to the soybean's ability to compensate for short durations of stress caused by environmental factors such as high or low temperature, water availability, or nutrient status. King et al. (2001) reported that application of Roundup Ultra® herbicide delayed nitrogen fixation and decreased nitrogen accumulation in some glyphosate-tolerant soybean cultivars. However, effects were only observed under drought conditions and at rates of glyphosate above the recommended label rates. The soybean yield was not affected. In a study which included four soybean varieties and four sites, Goos et al. (2002) reported that there was no indication Roundup Ultra herbicide inhibited nitrogen fixation, except that in one soybean variety at one site there was a small reduction in ureides, the principal products of nitrogen fixation in shoots of soybean. At recommended use rates, the application of glyphosate-based herbicides in the glyphosate-tolerant soybean production system is not expected to negatively affect soil fertility, nodule formation, or nitrogen fixation.

5. Transport through the Soil – Surfactant: Available data also suggest that the POEA surfactant used in Roundup agricultural herbicides binds strongly to soil (estimated soil organic carbon-water partition coefficient (Koc) values range from 2500 to 9600[49]) and undergoes microbial degradation with an estimated half-life of less than 14 days[50]. POEA is rapidly partitioned (half-life of 13 to 18 hours) from water to sediment in a water / sediment study (Wang et al., 2005). The rapid partitioning of the POEA surfactant to soil sediment combined with the high Koc values indicates that the surfactant will be tightly bound to the soil. The Groundwater Ubiquity Score, GUS, is an index that indicates the potential for compounds to leach from soil into groundwater, based on their half-life and Koc (Gustafson, 1989). Using an estimated half-life of 14 days and a Koc of 2500 as conservative estimates of the rate of degradation and binding to soil, the GUS index for the POEA surfactant is 0.69. According to the GUS movement ranking, this GUS index indicates that POEA has a very low potential to leach to groundwater.

® Roundup Ultra is a resistered trademark of Monsanto Technology LLC.

[49] Estimated from the partition ratio between water and sterile soil as reported in the POEA soil degradation study, Marvel et al., 1974.

[50] Marvel, J.T., Brightwell, B.B. and Suba, L.A. 1974. G3780A Surfactant: Biodegradation, plant uptake and ^{14}C Distribution. Monsanto Company Report No. 321

Attachment 1. Appendix O. U.S. Glyphosate Tolerances for Food & Feed Commodities (40 CFR § 180.364)

Commodity	Parts/million	Commodity	Partss/million
Acerola	0.2	Durian	0.2
Alfalfa, seed	0.5	Egg	0.05
Almond, hulls	25	Epazote	1.3
Aloe vera	0.5	Feijoa	0.2
Ambarella	0.2	Fig	0.2
Animal feed, nongrass, group 18	400	Fish	0.25
Artichoke, globe	0.2	Flax, meal	8.0
Asparagus	0.5	Flax, seed	4.0
Atemoya	0.2	Fruit, citrus, group 10	0.5
Avocado	0.2	Fruit, pome, group 11	0.2
Bamboo, shoots	0.2	Fruit, stone, group 12	0.2
Banana	0.2	Galangal, roots	0.2
Barley, bran	30	Ginger, white, flower	0.2
Barley, grain	20	Goat, kidney	4.0
Beet, sugar, dried pulp	25	Goat, liver	0.5
Beet, sugar, roots	10	Gourd, buffalo, seed	0.1
Beet, sugar, tops	10	Governor's plum	0.2
Berry group 13	0.2	Gow kee, leaves	0.2
Betelnut	1.0	Grain, aspirated fractions	100
Biriba	0.2	Grain, cereal, forage, fodder and straw, group 16, except corn forage	100
Blimbe	0.2	Grain, cereal, group 15, except barley, field corn, grain sorghum, oat and wheat	0.1
Borage, seed	0.1	Grape	0.2
Breadfruit	0.2	Grass, forage, fodder and hay, group 17	300
Cacao bean	0.2	Guava	0.2
Cactus, fruit	0.5	Herbs subgroup 19A	0.2
Cactus, pads	0.5	Hog, kidney	4.0
Canistel	0.2	Hog, liver	0.5
Canola, meal	15	Hop, dried cones	7.0
Canola, seed	10	Horse, kidney	4.0
Cattle, kidney	4.0	Horse, liver	0.5
Cattle, liver	0.5	Ilama	0.2
Chaya	1.0	Imbe	0.2
Cherimoya	0.2	Imbu	0.2
Citrus, dried pulp	1.5	Jackfruit	0.2
Coconut	0.1	Jaboticaba	0.2
Coffee, bean	1.0	Jojoba, seed	0.1
Corn, field, forage	6.0	Juneberry	0.2
Corn, field, grain	1.0	Kava, roots	0.2
Cotton, gin byproducts	175	Kenaf, forage	200
Cotton, undelinted seed	35	Kiwifruit	0.2
Cranberry	0.2	Lesquerella, seed	0.1
Crambe, seed	0.1	Leucaena, forage	200
Custard apple	0.2	Lingonberry	0.2

Commodity	Parts/million	Commodity	Parts/million
Date	0.2	Longan	0.2
Dokudami	2.0	Lychee	0.2
Mamey apple	0.2	Sapote, black	0.2
Mango	0.2	Sapote, mamey	0.2
Mangosteen	0.2	Sapote, white	0.2
Marmaladebox	0.2	Sesame, seed	0.1
Meadowfoam, seed	0.1	Sheep, kidney	4.0
Mioga, flower	0.2	Sheep, liver	0.5
Mustard, seed	0.1	Shellfish	3.0
Noni	0.20	Sorghum, grain, grain	15
Nut, pine	1.0	Soursop	0.2
Nut, tree, group 14	1.0	Soybean, forage	100
Oat, grain	20	Soybean, hay	200
Okra	0.5	Soybean, hulls	100
Olive	0.2	Soybean, seed	20
Oregano, Mexican, leaves	2.0	Spanish lime	0.2
Palm heart	0.2	Spearmint, tops	200
Palm heart, leaves	0.2	Spice subgroup 19B	7.0
Palm, oil	0.1	Star apple	0.2
Papaya	0.2	Starfruit	0.2
Papaya, mountain	0.2	Stevia, dried leaves	1.0
Passionfruit	0.2	Strawberry	0.2
Pawpaw	0.2	Sugar apple	0.2
Pea, dry	8.0	Sugarcane, cane	2.0
Peanut	0.1	Sugarcane, molasses	30
Peanut, hay	0.5	Sunflower	85
Pepper leaf, fresh leaves	0.2	Sunflower, seed	0.1
Peppermint, tops	200	Surinam cherry	0.2
Perilla, tops	1.8	Tamarind	0.2
Persimmon	0.2	Tea, dried	1.0
Pineapple	0.1	Tea, instant	7.0
Pistachio	1.0	Teff, grain	5.0
Pomegranate	0.2	Ti, leaves	0.2
Poultry, meat	0.1	Ti, roots	0.2
Poultry, meat byproducts	1.0	Ugli fruit	0.5
Pulasan	0.2	Vegetable, leafy, brassica, group 5	0.2
Quinoa, grain	5.0	Vegetable, bulb, group 3	0.2
Rambutan	0.2	Vegetable, cucurbit, group 9	0.5
Rapeseed, meal	15	Vegetable, foliage of legume, except soybean, subgroup 7A	0.2
Rapeseed, seed	10	Vegetable, fruiting, group 8	0.1
Rose apple	0.2	Vegetable, leafy, except brassica, group 4	0.2
Safflower	85	Vegetable, leaves of root and tuber, group 2, except sugar beet	0.2
Safflower, seed	0.1	Vegetable, legume, group 6, except soybean	5.0
Salal	0.2	Vegetable, legume, group 6 except soybean and pea,dry	5.0

Commodity	Parts/million	Commodity	Parts/million
Sapodilla	0.2	Vegetable, root and tuber, group 1, except sugar beet	0.2
Wasabi, roots	0.2	Wheat, grain	5.0
Water spinach, tops	0.2	Wheat, middlings	20
Watercress, upland	0.2	Wheat, shorts	20
Wax jambu	0.2	Yacon, tuber	0.2
Wheat, bran	20		

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Appendix P. Petitioner's Environmental Assessment

P.A. Background

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. Additionally, USDA-APHIS must comply with the National Environmental Policy Act (NEPA) when making the decision whether to grant deregulated status to MON 87705. Numerous field trials conducted in the U.S. under APHIS notifications and permits since 2005 have included MON 87705 as test material. Information has been collected from these field trials, other tests, and the literature to assess whether the improved fatty acid profile through suppression of *FATB* and *FAD2* RNAs, expression of the CP4 EPSPS enzyme to confer glyphosate tolerance and/or the plant transformation process has altered MON 87705 in any way that would impart plant pest characteristics or cause significant environmental impacts, including cumulative impacts. The purpose of this section is to provide relevant, trait-specific information regarding the potential for reasonably foreseeable, significant environmental impacts.

An analysis of the potential impact of deregulation of MON 87705 on current soybean agronomic production systems, and related activities such as soybean processing, food and feed uses as well as marketing of soybean and soybean products is presented in this section. Factors evaluated as part of the assessment include potential impacts to:

- land use patterns, non-agricultural lands, farming practices, commodity and specialty soybean production,
- marketability of soybean seed for planting and seed for specialty and commodity markets, and
- public health, non-target organisms, threatened or endangered species, and biodiversity.

The analysis conducted considers current conditions, the potential for deregulation of MON 87705 to impact these conditions, and potential cumulative impacts. In most cases, there are no impacts to current conditions (e.g., no differences between deregulation of MON 87705 versus continuing to regulate). Where differences were noted, these differences are described and their significance evaluated.

P.B. Purpose and Need

Monsanto Company (Monsanto) is submitting to APHIS this petition for the determination of nonregulated status for MON 87705 plants genetically enhanced to suppress the endogenous *FATB* and *FAD2* RNA in the developing soybean seed; thereby improving the fatty acid profile to contain lower saturated fat, higher oleic, and lower polyunsaturated fatty acid levels. MON 87705 soybean oil also has improved heat and oxidative stability and, due to the lower levels of saturated fats, a healthier profile relative to conventional soybean oil. MON 87705 also expresses CP4 EPSPS protein throughout the plant conferring tolerance to glyphosate, which is the active ingredient in the Roundup family of agricultural herbicides.

Initially the U.S. market for soybean oil was severely limited by the presence of undesirable off-flavors and odors. The double bonds in the polyunsaturated fatty acids

(PUFAs), particularly linolenic acid, are susceptible to oxidation resulting in the formation of "fishy" or acrid flavors and odors in soybean oil (Dutton et al., 1951). As a result, chemical hydrogenation was adopted to reduce the content of polyunsaturated fatty acids (Dutton, 1963; Okkerse et al., 1967). At first, hydrogenated vegetable oils, including hydrogenated soybean oil, were viewed as viable alternatives to animal fats and the high-in-saturated-fats tropical oils such as palm oil and coconut oil. However, in the 1990s, nutrition research showed that the *trans* fatty acids in hydrogenated oils created by the hydrogenation process had negative health consequences (Judd et al., 1994; Mensink and Katan, 1990; Zock and Katan, 1992). By 2006, the U.S. FDA had issued a regulation obligating food manufacturers to declare the *trans* fatty acid content of their product on nutrition labeling (FDA, 2006). As a result, soybean oil per capita consumption in U.S. has been declining since 2006 (Soyatech, 2008).

MON 87705 soybean oil fatty acid profile provides important new formulation options for food companies interested in the development of lower saturated fat food products to support heart health. Because MON 87705 soybean oil has a reduced level of PUFAs, it has higher oxidative stability without the need for hydrogenation, and has lower levels of saturated fats compard to commodity soybean. Saturated fats, notably palmitic acid (16:0), contribute to cardiovascular disease and other chronic diseases in epidemiological trials (Hu et al., 1997). As a result, reducing saturated fat levels in soybean oil can positively impact the goal of keeping human dietary consumption of saturated fats below 10% of their total energy intake (USHHS, 2005).

As mentioned, a reduction in saturated fats and increased oxidative stability in MON 87705 soybean oil contribute to an important new formulation option for food companies. The uses of soybean oil for biodiesel and other industrial applications is also enhanced by virtue of a modified oil profile similar to that found in MON 87705 soybean oil. Low saturated fats and high (>70%) oleic acid levels are key attributes for vegetable oils targeted for biodiesel and industrial uses because these characteristics are vital to improved cold weather performance, improved stability, and reduced nitrous oxide emissions (Knothe, 2005; Graef et al., 2009).

Therefore, the decrease in saturated fats and PUFAs (17% vs. 60% FA), in MON 87705 soybean oil provides important options for food companies to develop lower saturated fat foods with greater food functionality. In addition, MON 87705 soybean oil attributes provide key enhancements for biodiesel and industrial applications.

P.C. Soybean Production

The following section describes the setting for the proposed deregulation and provides the context for evaluating the intensity of the impact due to USDA-APHIS granting deregulated status to MON 87705. 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 27 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, 2006).

In recent years, there has been an increased demand by consumers and food processers for soybean and other oilseed crops that have specific physical or chemical characteristics

to meet specific food or feed needs. Approximately 12% of soybean grown is specialty soybean produced for a specific market or use (United Soybean Board at http://www.unitedsoybean.org), including high protein, tofu, nonbiotechnology-derived, organic and high oleic and low linolenic acid content. The specialty, value-added product may be the whole bean or a fraction such as the oil. The vast majority of soybean in the U.S. is grown for animal feed and is fed as soybean meal. During processing, soybean oil is extracted to produce crude soybean oil and defatted soybean meal. According to a 1999 American Soybean Association sponsored study the oil content of soybean meal after extraction is relatively low, between 1-3%. (http://www.soymeal.org/worldlitarticles_new/globalsmsampling.html).

Specialty soybean varieties are specified by buyers and end-users of soybean for production, and premiums are paid for delivering a product that meets purity and quality standards for the soybean variety. Product differentiation and market segmentation in the specialty soybean industry includes mechanisms to keep track of the soybean (traceability), methods for identity preservation (IDP), such as quality assurance processes (e.g., ISO9001-2000 certification), as well as contracts between growers and buyers that specify delivery agreements. Ultimately, the amount of planted acreage will be driven by consumer preferences based on the demand for the oil produced by MON 87705. On the basis of predicted demand for food and feed applications, and the additional health benefits and functionality of MON 87705 soybean oil, it is initially expected to be a specialty soybean produced for its premium oil. This premium is determined by the increased cost of production and distribution throughout the supply chain (mostly IDP costs) and market demand for the enhanced composition, which in the case of MON 87705 soybean oil is an improved fatty acid profile that is higher in oleic acid and lower in saturated fats than commodity soybean oil

Data in Section VII of this petition show that, aside from the improved fatty acid profile present in the oil, the other processed fractions (meal, lecithin, and protein isolate) from MON 87705 seed are compositionally and nutritionally equivalent to nonbiotechnology-derived conventional processed fractions.

This deregulation is being sought in an environment that has rapidly adopted biotechnology-derived soybean varieties (James, 2007). Thirteen different biotechnology-derived soybean events have been granted nonregulated status by USDA since 1994 (www.aphis.usda.gov). In particular, 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-NASS, 2008). Thus, soybean breeders, seed manufactures, and soybean producers have developed practices and systems that allow for the concurrent breeding and manufacture of biotechnology-derived and specialty soybean and are capable of breeding and manufacturing seed, and producing harvested seed to meet the needs of various markets.

Traditional plant breeding has been used to alter the fatty acid profiles of oil producing plants such as soybean. For example, soybean varieties with lower levels (1-3%) of the polyunsaturated linolenic fatty acid have recently entered the market (Fehr, 2007) and programs to develop high oleic soybean through conventional breeding have resulted in soybean varieties with an oleic content of >70% (Alt et al., 2005). More recently soybeans with alterations in the fatty acid profile from genetic modifications have either been submitted to or deregulated by USDA-APHIS (Table P-1).

Table P-1. Deregulated or Submitted Biotechnology-derived Soybean Products with Altered Oil Profiles

Phenotype	ID Code(s)	Institution	Date Deregulated
Altered Oil Profile	MON 87769	Monsanto	Submitted
High Oleic Acid	DP-3Ø5423-1	Pioneer	Submitted
Altered Oil Profile	G94-1, G94-19, G-168	DuPont	May, 1997

Source: http://www.aphis.usda.gov/brs/not_reg.html

The MON 87705 soybean oil improved fatty acid profile provides important new formulation options for food companies interested in the development of lower saturated fat food products to support heart health. Conventional soybean oil typically contains 60-65% polyunsaturated fatty acids. Because MON 87705 soybean oil has a reduced level of polyunsaturated fatty acids, it has higher oxidative stability without the need for hydrogenation, and has lower levels of saturated fats compard to commodity soybean. The low saturated fat/high oleic oil produced from MON 87705 soybeans is suitable for use in products such as vegetable oils, salad dressings, margarine, etc., or in other applications for which commodity soybeans are used. Additionally, the use of soybean oil for biodiesel and other industrial applications also is enhanced by virtue of an improved fatty acid profile found in MON 87705 soybean oil.

P.D. Potential Environmental Impacts

The soybean production environment and MON 87705 are described in detail throughout this petition. The discussion of impacts below is focused on specific areas of the production environment and related activities including land use patterns, farming practices, commercial activities such as seed production and marketing of soybean and impacts to the quality of agricultural products.

P.D.1. Impacts on Land Use

Soybean is grown as a commercial crop on over 75 million acres in at least 27 states in the U.S. (USDA-NASS, 2009a). Soybean acreage in the past five years has been relatively stable varying from 64.7 million to 75.7 million acres with a 10-year average of 73.3 million acres (Table IX-1). Fluctuations in soybean acreage are due to environmental, agronomic and economic factors[1], as well as government programs such as the crop reserve program (CRP) or ethanol mandates imposed by the U.S. government. 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 87705 is not expected to differ from typical soybean cultivation practices. The improved fatty acid profile trait provides growers with an option to produce a value added soybean and potentially greater profitability from their farming operation (see Section IX). Additionally, canola and

[1] 2008 NASS crop acreage report [http://www.nass.usda.gov/Newsroom/2008/06_30_2008.asp] accessed 6/2009

sunflower production acreage has fluctuated due to complex market demands. During the period of 1998 through 2008, canola acreage has ranged from 865,000 to 1,555,000 acres. Likewise, during the same period, sunflower acreage fluctuated between 246,869 and 669,338 acres[2]. MON 87705 will likely be used in common rotations on land previously used for agricultural purposes.

There is no indication that the introduction and widespread adoption of biotechnology-derived crops in general has resulted in a significant change to the total U.S. acreage devoted to agricultural production. 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 at 326 million acres since the introduction of biotechnology-derived crops in 1996[3].

Granting deregulated status to MON 87705 is not expected to significantly alter commercial soybean cultivation in terms of agricultural inputs and production management (see Section IX). Currently, biotechnology-derived herbicide tolerant soybean is planted on 92% of the soybean acreage (USDA-NASS, 2008) and the Roundup Ready soybean system has become the standard weed control program in U.S. soybean production. Therefore the presence of the herbicide tolerance trait in MON 87705 does not offer a new or different incentive to growers that would be expected to significantly alter commercial soybean cultivation.

A possible cumulative impact from the introduction of herbicide-tolerant soybean varieties is the increase in the practice of no-till or conservation tillage by U.S. growers. In 1995, before the introduction of glyphosate-tolerant soybeans, approximately 27% of the U.S. soybean acres used no-till production. By 2004, no-till acres increased to 36% of the total soybean acres (Sankula, 2006). A few states provide statistics on the adoption of no-till acres in their state. In 2006 in Illinois, no-till farming was used on 51% of the soybean acres. The University of Illinois Extension Service attributed that figure to the fact that 90% of the state's acres were planted to glyphosate-tolerant varieties, along with other factors, such as high fuel prices, improved equipment, higher yields using no-till practices, and better grower awareness of the advantages to soil and water quality from no-till farming[4]. For an overview of the cumulative impacts (including tillage) on land use from deregulation of glyphosate-tolerant crops see Appendix N.

Additionally, because MON 87705, with the exception of the improved fatty acid profile, is phenotypically and agronomically equivalent to commercial soybean varieties, there is no expectation that introduction of MON 87705 will significantly alter the range of commercial soybean cultivation. Although it is not known how many acres will be planted to MON 87705, it is expected that this product and/or subsequent combinations

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[2] Comparisons of canola and sunflower acreage established from databases at http://usda.mannlib.cornell.edu/usda/current/htrcp/

[3] Calculated from database at http://usda.mannlib.cornell.edu/usda/current/htrcp by comparing the total acres from 1983 to 1995 and from 1996 to 2007.

[4] University of Illinois Extension Servive News Release: No-till is now the "Conventional" Tillage System for Illinois Farmers; http://web.extension.uiuc.edu/state/newsdetail.cfm?NewsID=4991

of this product with other biotechnology-derived or conventional soybean varieties potentially could be grown on the majority of U.S. soybean acres.

Because of the similar oil profiles, it is possible that the introduction of MON 87705 and/or subsequent combinations of MON 87705 with other biotechnology-derived and conventional soybean varieties could impact demand for oil from other oilseed crops such as canola and sunflower. The decision to plant a crop is based on many factors including demand, input costs, environmental conditions and geography. Soybean acreage does not tend to overlap land typically used for canola and sunflower production in the U.S. (Figure P-1). Additionally, canola and sunflower production acreage has fluctuated due to complex market demands. During the period of 1998 through 2008, canola acreage has ranged from 865,000 to 1,555,000 acres. Likewise, during the same period, sunflower acreage fluctuated between 246,869 and 669,338 acres[5]. Because of these geographic differences and complex market dynamics, it is unlikely that the introduction of MON 87705 will significantly impact the planting of other oil producing crops and should not result in significant changes to land use patterns. If, however, the demand for canola and sunflower oil were to decline, growers have the option to grow non-oil producing crops such as corn and wheat on acreage currently dedicated to canola and sunflower.

Growers make planting decisions for all crops based on market demand, commodity prices, and the agricultural needs and practices specific to their acres. Because of this, it is not anticipated that the introduction of MON 87705 will result in a significant impact to canola and sunflower growers or to agricultural land use in those geographic areas where canola and sunflower are currently grown.

[5] Comparisons of canola and sunflower acreage established from databases at http://usda.mannlib.cornell.edu/usda/current/htrcp/

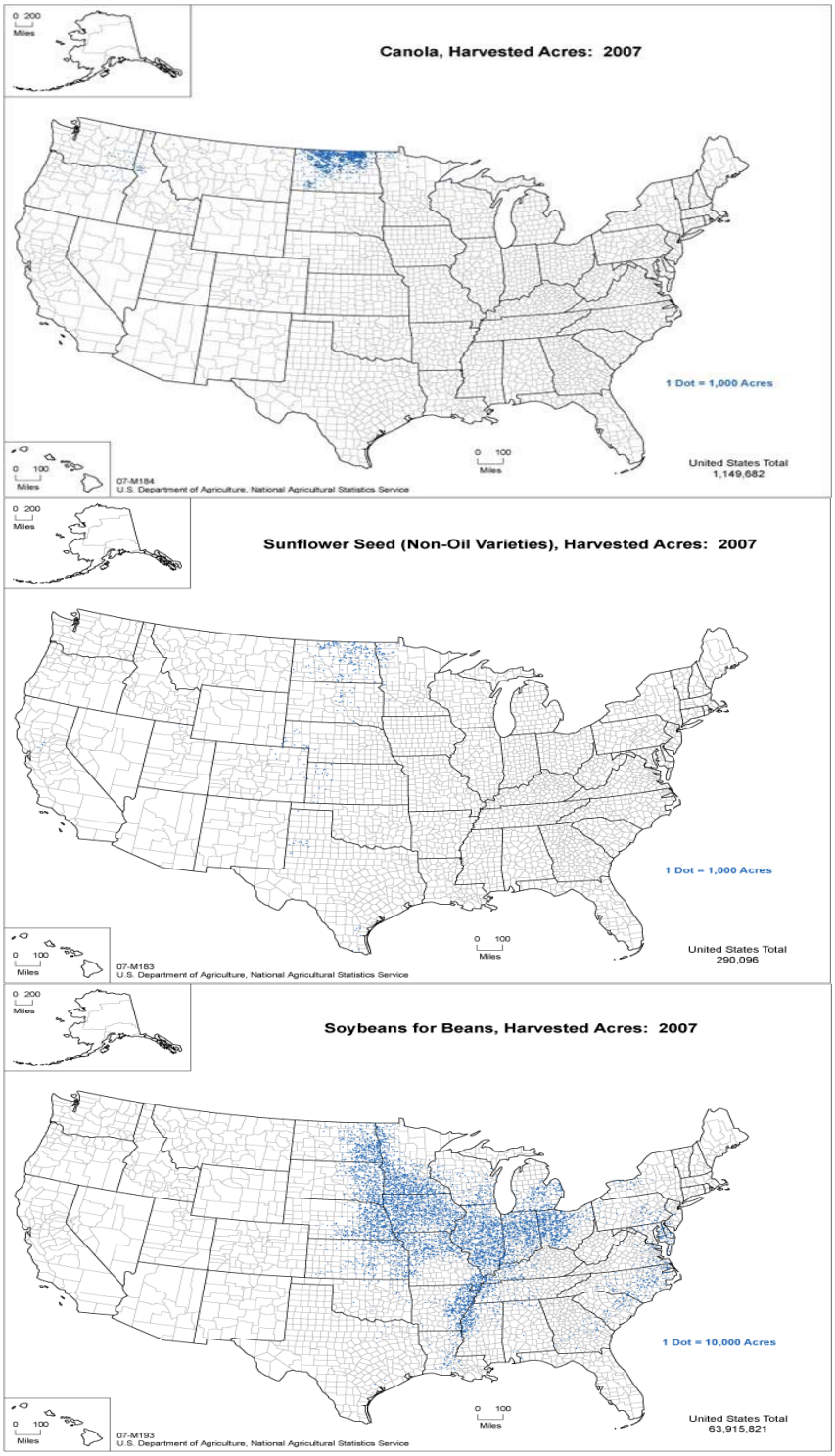

Figure P-1. Harvested U.S. Acres of Canola, Sunflower and Soybean
Source:
http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Ag_Atlas_Maps/Crops_and_Plants/index.asp

P.D.2. Potential for Non-crop and Non-agricultural Impacts

Soybean (*Glycine max*) does not grow 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 suppression of the *FATB* and *FAD2* RNAs resulting in an improved fatty acid profile, MON 87705 is similar to other commercial soybean varieties currently grown in the U.S. in that both MON 87705 and 92% of commercially grown soybean have the *cp4 epsps* gene that confers tolerance to the herbicide glyphosate (USDA-NASS, 2008). Therefore, MON 87705 is expected to have no significant impact to non-agricultural lands and aquatic systems beyond those related to commercially grown soybean. There are no changes in agricultural inputs needed to produce a crop from MON 87705 relative to commodity and specialty soybean and specifically no altered pest or disease susceptibility. Hence, pesticide applications on MON 87705 that may result in drift impacting non-crop or non-agricultural lands would be comparable to those used on conventional or currently available soybean varieties.

Because MON 87705 produces the CP4 EPSPS protein conferring tolerance to the herbicide glyphosate it is anticipated that glyphosate will be used to control weeds in fields planted with MON 87705. Glyphosate has been approved for use by the U.S. Environmental Protection Agency and food and feed tolerances have been established in the U.S. for its residues, since 1979. Glyphosate has a complete and comprehensive regulatory data base (toxicity, environmental fate, and ecological toxicity) that has been evaluated by EPA and it has been determined that "glyphosate will not pose unreasonable risks or adverse effects to humans or the environment" (EPA, 2002). More information on the impacts of glyphosate to animal and plant communities and threatened and endangered species may be found in Section P.D.7 of this appendix and Appendix O. Likewise a comprehensive discussion concerning the development of glyphosate resistant weeds may be found in Appendix L.

P.D.3. Potential Impacts to Agricultural Practices

MON 87705 has been shown to be no different from conventional soybean in its agronomic and ecological characteristics (see Sections VII, VIII, IX and X), and has the same levels of resistance to insects and diseases as commercial soybean. A summary of agronomic practices for soybean production is presented in Section IX of this petition. Because glyphosate-tolerant soybean is already grown on 92% of U.S. soybean acres (USDA-NASS, 2008), no significant impact is expected from the introduction of MON 87705 on current cultivation practices, including seeding, tillage, fertilizer and pesticide applications and crop rotation practices for soybean (Section IX). An analysis of expected impact to agricultural practices for commodity seed and certified seed production as well as potential impacts to specialty soybean production are discussed below.

Potential Impact to Soybean Commodity and Specialty Soybean Production

Monsanto will seek regulatory approval for MON 87705 and its combinations, where required, with other biotechnology-derived soybean varieties in all key soybean import countries with a functioning regulatory system to to support the flow of international trade. MON 87705 would provide food manufacturers and consumers with a soybean oil having an improved fatty acid profile and greater thermal and oxidative stability for multiple uses. It would also provide growers with a higher value crop resulting in greater profit potential for U.S. soybean producers. Soybean growers and processors are accustomed to accommodating a diversity of specialty soybean varieties in their normal handling of commodity soybean. Currently specialty soybean varieties are produced on approximately 12% of the U.S. soybean acreage (see Section IX.B.2). Soybean growers have demonstrated an ability to provide customers with their soybean product of choice. As discussed in Section IX, MON 87705 will be produced using IDP practices consistent with those used for other specialty soybean varieties. MON 87705 will be segregated from commodity soybean by producers as part of their contract and to maintain the value of the crop. Because soybean is primarily a self-pollinated crop with minimal gene movement and due to the identity preservation production system, no significant impact to commodity or other specialty soybean production is anticipated, should APHIS grant nonregulated status to MON 87705.

No cumulative impacts to commodity or other specialty soybean production are expected from deregulation. Soybean producers have demonstrated their ongoing ability to produce multiple specialty soybean varieties in a diverse marketplace.

Potential Impact to Certified Seed Production

Certified seed production is a carefully managed process (Section IX.B.4). MON 87705 is not expected to impact certified seed production practices or production of other certified commodity or specialty soybean seed for reasons described in this section.

If MON 87705 is deregulated, seed production could occur within production systems already developed by seed producers for certified seed varieties (see Section X). MON 87705 has been thoroughly characterized and (with the exception of the modified fatty acid profile) is not agronomically or phenotypically different from currently commercial soybean. 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 (Section IX.B.4). Soybean is a highly self-pollinated species that exhibits very low levels of outcrossing. Numerous studies on soybean cross-pollination have been conducted, and the published results, with and without supplemental pollinators, are summarized in Table X-1. Under natural conditions, cross-pollination among adjacent plants in a row or among plants in adjacent rows ranged from 0 to 6.3%. In experiments where supplemental pollinators (usually bees) were added to the experimental area, cross-pollination ranged from 0.5 to 7.74% in adjacent plants or adjacent rows. However, cross-pollination does not occur at these levels over long distances. Cross-pollination rates decrease to less than 1.5% beyond one meter from the pollen source, and rapidly

decrease with greater distances from the source. The following cross-pollination rates at extended distances have been reported: 0.02% at 8.2 m of separation (Caviness, 1966), 0.05% at 5.4 m (Ray et al., 2003), and 0% at 6.5 m (Abud et al., 2003). Hence, certified soybean seed producers can and have effectively implemented practices (e.g., 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.

No cumulative effects are anticipated to certified seed production from deregulation of MON 87705. The use of management practices that prevent trait movement and comingling of soybean varieties has resulted in production of soybean varieties with improved genetics over time. As a result of the implementation of these management practices, growers today can choose from numerous varieties of soybean including those used to produce commodity and specialty soybean varieties.

Potential Impacts to Organic Soybean Production

Organic soybean markets typically enjoy a market premium offsetting the additional production and record-keeping costs associated with this production method (see Section IX.B.2). 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 or products of excluded methods from adjoining land that is not under an organic production management plan. Organic production operations must also develop and maintain an organic production system plan approved by an accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition of the use of excluded methods. Excluded methods include a variety of methods used to genetically engineer organisms or influence their growth and development by means that are not possible under natural conditions or processes. The use of biotechnology such as that used to produce MON 87705 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 certifies that organic production and handling processes have been followed, not that the product itself is "free" from any particular substance.

Production systems designed prior to the introduction of MON 87705 or even prior to the introduction of biotechnology-derived soybean have allowed for production of soybean to meet varied customer demands. In addition to the market segments that 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 (Cui et al., 2004). The choice to grow biotechnology-derived, organic or conventional soybean depends on many factors and the dynamics of the marketplace. 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 87705.

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 87705 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 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 level of outcrossing. Hence, organic or conventional soybean producers can and have effectively implemented practices (e.g., isolation during the growing season, equipment cleaning during harvest, and post-harvest separation of harvested seed) that allow them to avoid the presence of biotechnology-derived soybean and maintain organic or conventional production status.

Currently, biotechnology-derived herbicide tolerant soybean is planted on 92% of the soybean acreage (USDA-NASS, 2008) and the Roundup Ready soybean system – that is, planting Roundup Ready soybean and applying glyphosate in crop – has become the most widely used weed control program in U.S. soybean production. 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 using these production practices or 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 P-1). Additional information on organic seed sources is provided though the U.S. Agricultural Marketing Service (AMS) at:

www.ams.usda.gov. Thus, growers have a choice in the soybean variety they plant, and this is not expected to change with the introduction of MON 87705.

Buyers recognize that where there are biotechnology-derived crop varieties on the market, as with soybean, a guarantee that a commodity crop is 100% "free" of biotechnology-derived material is not feasible based on the limitations of testing and sampling methodology (Born, 2005). While the presence of a biotechnology-derived product like MON 87705 is unlikely in instances where producers utilize production practices designed to avoid biotech presence, in some instances buyer allowances between 0.1 to 5% biotechnology-derived commodity soybean in organic soybean are specified. 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 commodity or specialty soybean is 100% free of biotechnology-derived material. Thus, they have set allowable tolerances for this material in conventional products to support food labeling 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 may trigger special labeling. Thus, *de minimis* levels of approved biotechnology-derived soybean mav be allowable in certified organic or conventional soybean.

Table P-2. Organic and Conventional Soybean Seed Sources

Organic Soybean Seed Sources*:	**Conventional Soybean Seed Sources**
Albert Lea Seed House	AgVenture Seeds (modified oil)
Blue River Hybrids	Campbell Seed (modified oil)
Golden Grains	Becks Hybrids (food grade)
Great Harvest Organics	Monsanto (Asgrow)
Greis Seed Farm	Schillinger Seed
Lancaster Ag Products	Pioneer
Lawler Farm Center	Soy Genetics
Prairie Gold Seeds	Stewart Seed (modified oil)
Superior Organic Grains, Ltd	Stine Seed
Walter Seed and Honey Co	Syngenta - multiple brands
	Terral Seed
	Various State Crop Improvement Organizations

* From: www.organicgrains.ncsu.edu

P.D.4. Potential Impacts to Raw or Processed Agricultural Commodities

Within this petition, extensive data have been presented relating to plant growth parameters, disease susceptibility, insect susceptibility, and forage and seed composition of MON 87705 compared to conventional soybean varieties. These data indicate that there are no biologically relevant differences between MON 87705 and conventional soybean, except for the deliberate change in the fatty acid profile of the oil and its glyphosate tolerance. Biotechnology-derived soybean varieties like MON 87705 undergo a voluntary food and feed consultation process with the FDA prior to release on the market. Monsanto will complete this consultation process prior to a commercial introduction of MON 87705.

From a grain quality standpoint (e.g., foreign material, damaged grain, etc; see www.gipsa.usda.gov for soybean grain standards), the soybean produced from MON 87705 varieties is expected to be of comparable quality to soybean commercially produced in the U.S. for commodity markets based on the data presented herein. The low saturated fat, high oleic acid oil produced from MON 87705 soybean is suitable for use in any application for which commodity soybean oil is currently used, including products such as vegetable oils, salad dressings, margarine, etc.

As described elsewhere in this petition, MON 87705 and the low saturated fat, high oleic acid soybean oil produced from MON 87705 will be produced and processed utilizing an identity preservation (IDP) system to capture the food quality value of the oil. Because other low saturated fat, high oleic acid oils such as canola and sunflower are already present in the commodity stream and are routinely handled by processors using standard industry processing methods as described in Appendix K, the introduction of MON 87705 will require no new inventions or processes. Processors routinely perform analyses for oil quality and fatty acid composition that enable them to capture the value of specialty soybean oils, to determine the appropriate customers and uses for various soybean and other oils, and to create the appropriate and requested blends of such oils for particular uses.

Soybean is typically processed into two major fractions, the oil and meal, and several minor fractions including lecithin and protein isolate. The MON 87705 oil fraction will have the intended low saturated fat, high oleic acid profile, and will be produced and utilized within an IDP system. With the exception of low level residual oil, the other processed fractions derived from MON 87705 are compositionally equivalent to processed fractions from commodity soybean, and will be utilized in a manner similar to commodity soybean processed fractions (see Section VII.C). The meal, and other non-oil processed fractions are intended to be distributed into the commodity stream and will be marketed and sold in the commodity market. Because of their compositional equivalence, it is not anticipated that deregulation of MON 87705 will have a significant impact on these commodity soybean processed fractions.

During the development of MON 87705, Monsanto has engaged in dialogue with relevant grain handlers, processors and food companies regarding the uses and

applications of low saturated fat, high oleic soybean oil. This dialogue has contributed to the understanding of the processing and use of MON 87705 soybean and the resulting oil and other fractions, and to the market and trade assessments set forth in this petition. Before implementing an IDP system for MON 87705-derived varieties, Monsanto will have additional dialogue with relevant stakeholders in the value chain to discuss and understand the potential impacts on commodity oils and processed soybean products from the IDP production, handling and use of MON 87705 soybean and its low saturated fat, high oleic soybean oil. Based on that dialogue and any resulting refinements to the market and trade assessments, Monsanto will take the following steps, where applicable and appropriate:

1. refine plans for production, handling and processing of MON 87705-derived soybean varieties and the low saturated fat, high oleic soybean oil (the IDP system) to address valid concerns raised by the stakeholders
2. assess potential impacts on processed soybean products due to the presence of low saturated fat, high oleic soybean oil in commodity oil, and collaborate with stakeholders to support the evaluations needed to assess when potential impacts might occur, what processes may be necessary to mitigate any potential negative impacts, and what potential positive impacts may occur based on the improved oil profile
3. engage in industry outreach and education regarding the MON 87705 soybean and low saturated fat, high oleic soybean oil IDP system, including product handling and use
4. make available prior to commercialization a detection method or testing regime that enables identity verification of MON 87705 for its intended use

Based on our market and trade assessments, because MON 87705 soybean is appropriate for all the same uses as commodity soybean, the only likely potential impact on raw or processed commodities in the event MON 87705-derived varieties reach the commodity channel would be a reduction in saturated and polyunsaturated fats in commodity oil. This reduction in saturated and polyunsaturated fats would improve the functionality of the oil and not impact the suitability of the soybean oil for anticipated end uses. The only impact would be an improvement in the nutritional profile of the oil relative to commodity soybean oil. Based on this analysis, there would be no significant impacts on raw or processed soybean commodities due to USDA-APHIS granting nonregulated status to MON 87705.

Based on this assessment, Monsanto does not anticipate any significant cumulative impacts on either raw or processed soybean commodities resulting from the deregulation of MON 87705. Food ingredient and food manufacturers routinely process, manage, handle and blend a wide variety of specialty oils derived from oilseed crops (including other modified oils produced by conventionally bred varieties or previously deregulated events) with no adverse or cumulative effects on their manufacturing processes or on the quality of their products. Similarly, food manufactures will integrate low saturated fat,

high oleic soybean oil into their existing handling and manufacturing process, and are expected to have the equivalent experience with low saturated fat, high oleic soybean oil, resulting in no adverse or cumulative effects due to USDA-APHIS granting deregulated status to MON 87705.

P.D.5. Potential Impacts on Commercial Use of Soybean

The decision to deregulate MON 87705 would allow for breeding of this product with conventional and previously deregulated biotechnology-derived soybean varieties of diverse genetic backgrounds. These varieties will include commercial varieties with low linolenic acid levels which can further enhance the oxidative stability of the soybean oil. In addition, MON 87705 will be combined using traditional breeding methods with other biotechnology-derived traits, including glyphosate tolerance (MON 89788), to deliver the best agronomic platform to farmers. It is expected that breeders and certified seed producers would use MON 87705 to develop varieties and to supply seed for planned commercial markets in the U.S. Monsanto anticipates that commercial use of MON 87705 will include export of soybean and soybean products, and has described import approval submission plans elsewhere in the petition. Monsanto's stewardship program for this product is described in Section IX.I.

There are no statistically significant differences observed in yield between MON 87705 and its conventional control (Table VIII-4). However, MON 87705 represents an additional trait to those commonly selected for by soybean breeders. Such traits, whether conventionally bred or biotechnology-derived, have the potential to impact yield gains (Fehr, 2007). Monsanto and its breeding company partners have aggressive breeding programs in place which utilize increased population size and yield testing, and advanced molecular selection tools. This program will ensure that MON 87705 yields are continually increasing comparable to conventional commodity soybean, thus providing a competitive commercial soybean alternative to U.S. growers.

P.D.6. Potential Impacts to Human Health and Safety

Most human interactions with soybean occur through agricultural production, industrial operations, or through consumption. Therefore, this health and safety discussion will focus on food and feed safety as well as safety of workers exposed to MON 87705. The low saturated fat, high oleic soybean oil derived from MON 87705 soybean oil is suitable for use in products such as vegetable oils, salad dressings, margarine, etc., or in any application for which commodity soybean oil is used.

Under the Federal Food, Drug, and Cosmetic Act (FFDCA), it is the responsibility of food and feed manufacturers to ensure that the products they market are safe and properly labeled. Food and feed derived from MON 87705 must be in compliance with all applicable legal and regulatory requirements. Biotechnology-derived crops for food and feed use undergo a voluntary consultation process with the FDA prior to release onto the market. Although a voluntary process, Monsanto routinely completes a consultation with

the FDA prior to placing a new biotechnology derived crop product on the market. A list of completed consultations on biotechnology-derived crop products is available at the FDA website.

Human Health

MON 87705 is genetically modified to suppress endogenous *FATB* and *FAD2* RNAs in the developing soybean seed, thereby improving the fatty acid profile to contain lower saturated fat, higher oleic and lower linoleic fatty acid levels. Therefore, the improved soybean oil from MON 87705 has enhanced heat and oxidative stability, and, due to the lower levels of saturated fats, a healthier fatty acid profile relative to conventional soybean.

MON 87705 contains only those fatty acids that are presently found in soybean oil. MON 87705 has a fatty acid profile that is comparable to commercial high oleic vegetable oils (e.g., high oleic canola, high oleic safflower, high oleic sunflower), traditional oils such as olive oil that has a long-history of consumption in the diet, and canola oil that obtained U.S. FDA GRAS status.

Extensive compositional analyses of forage and seed were conducted on samples from replicated, multi-site field trials to compare the composition of MON 87705 to a conventional soybean control and to commercially available soybean varieties (see Section VII for details). The compositional analyses of MON 87705 confirmed the intended changes to four major soybean oil fatty acids. All other components analyzed in MON 87705 seed and forage were either not statistically significantly different compared to a conventional control, or, if significantly different, were within the 99% conventional soybean tolerance interval with the exception of eicosenoic acid. This value (0.34% FA) fell outside the tolerance intervals. However, eicosenoic acid has a history of safe consumption at higher levels in other commonly consumed vegetable oils such as canola oil (4.3% FA), peanut oil (1.7% FA), high oleic safflower oil (0.5% FA) and high oleic sunflower oil (0.5% FA) (Codex 2005).

The Dietary Guidelines for Americans (USHHS, 2005) recommend that saturated fat intake should be kept below 10% of total daily caloric intake and below 7% for individuals with elevated low density lipoprotein (LDL) blood cholesterol. The Dietary Guidelines also recommend that most dietary fat should come from PUFA and MUFA sources, such as vegetable oils. When MUFAs, such as oleic acid, are substituted for saturated fat in the diet, LDL cholesterol decreases. Likewise, diets high in PUFAs, such as the omega-6 fatty acid linoleic acid, are associated with a favorable blood lipid profile, and diets that contain 5-10% of energy as omega-6 PUFAs may confer benefits on coronary artery disease mortality.

MON 87705 soybean oil contains approximately 6% saturated fatty acids, approximately 76% 18:1 oleic acid and approximately 10% 18:2 linoleic acids levels. The fatty acid profile of MON 87705 is similar to a variety of other widely consumed vegetable oils

such as canola and olive oils (Table P-3) for which the U.S. FDA has approved a qualified health claim related to coronary heart disease.[56] Fatty acid profiles similarly higher in oleic acid than conventional soybean oil are also found in other vegetable oils such as high oleic safflower and sunflower oils and in nuts such as filberts, almonds, pistachios and pecans (Gunstone, 1994). These edible fats and oils are available for consumption in a wide variety of products including shortenings, salad and cooking oils, margarine and mayonnaise.

Table P-3. Comparison of Fatty Acid Profiles from Plant Sources

Oil	%* Saturated Fat	%* Oleic Acid (18:1)	%* Linoleic Acid (18:2)	%* Linolenic Acid (18:3)	%* PUFAs
Canola	6	57	26	10	32
MON 87705 Soybean	6	76	10	7	17
Conventional Soybean	15	23	53	8	60
Olive	13	78	7	1	8
Palm	50	38	11	1	12
Coconut	92	6	2	0	2

Source: Padley et al., 1994.

*% of total fatty acids

A dietary assessment was conducted in which MON 87705 replaced conventional liquid soybean oil in salad dressings, margarine and spreads, mayonnaise and home use of liquid soybean oil. The assessment indicates that intake of the saturated fat (16:0 palmitic acid) would decrease from 14.4 g/day to 13.8 g/day representing a decrease of 3.8% on a mean per capita basis. A summary of this assessment can be found in Appendix M. A decrease in dietary saturated fat intake is consistent with current dietary recommendations (Lichtenstein et al., 2006; USHHS, 2005; WHO/FAO, 2003). There was an overall increase in the total daily intake of oleic acid from 27.3 g/day to 30.3 g/day representing an increase of 10.8% on a mean per capita basis. The oleic acid increase post-replacement is expected, since, similar to olive oil or canola oil, the oleic acid fraction in MON 87705 soybean oil is higher than the commodity soybean oil it replaced. Oleic acid is not an essential fatty acid and no recommended intake level has been set by the Institute of Medicine of the National Academies (IOM, 2002); however, as stated above, it is recognized that there is an inverse relationship between the intake of MUFAs like oleic acid and the total cholesterol (TC):HDL cholesterol (HDL-C) concentration ratio.

[56] http://www.cfsan.fda.gov/~dms/qhccanol.html, http://www.cfsan.fda.gov/~dms/qhcolive.html

In summary, the nutritional impact from the use of MON 87705 soybean oil in targeted foods under the intended conditions of use, is estimated to result in changes in fatty acid consumption that are in line with current dietary guidelines for fatty acid intake. Therefore, MON 87705 is regarded to be as safe and nutritious as conventional soybean for food use.

MON 87705 produces a lower saturated fat, high oleic soybean oil that has improved oxidative stability and, due to the lower levels of saturated fats, a healthier fatty acid profile relative to comodity soybean oil. As discussed previously, Monsanto will provide the U.S. FDA with information on identity, function, and characterization of the genes, including expression of the gene products. Additionally, information on the safety of the improved fatty acid profile in MON 87705 soybean oil, including a dietary risk assessment, will be provided to the U.S. FDA for evaluation including a dietary risk assessment. On the basis of the assessment of laboratory data and scientific literature, it is reasonable to conclude that MON 87705 is safe for food and feed use.

MON 87705 also expresses the CP4 EPSPS protein throughout the plant conferring tolerance to glyphosate, which is the active ingredient in the Roundup family of agricultural herbicides. It is structurally homologous to EPSPS proteins that are part of the amino acid synthesis pathway of all plants (Devine et al., 1993). The safety of any protein(s) newly introduced into a biotechnology-derived crop needs to be assessed (Delaney et al., 2008; ILSI, 2004). The safety of CP4 EPSPS protein present in biotechnology-derived crops has been extensively evaluated (Harrison et al., 1996). The U.S. EPA has also reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR § 174.523). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of other Roundup Ready crops in 1996.

Roundup Ready soybean is planted on 92% of U.S. soybean acreage (USDA-NASS, 2008). Consequently glyphosate is the most widely used herbicide in soybean. The toxicology of glyphosate has been extensively reviewed. A summary of food and feed tolerances, regulatory approvals with associated dietary exposure assessments and recent chronic and short-term aggregate risk assessments for glyphosate may be found in Appendix O.

Worker Safety

In the agricultural production of soybean, growers may be exposed to pesticides during application of chemicals to crops. Planting Roundup Ready soybean and applying glyphosate in crop has become the standard weed control program in U.S. soybean production. MON 87705 will share that Roundup Ready weed management system, so any adoption of MON 87705 by growers currently planting Roundup Ready soybean would not significantly change the commercial soybean agronomic practices, or use of

pesticides such as herbicides, associated with soybean production (see Section IX). Worker safety issues related to the use of pesticides during agricultural production of MON 87705 will remain the same. A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development, and does not bioaccumulate in mammals (Williams et al., 2000). This petition demonstrates that, other than the improved fatty acid profile of the soybean oil and tolerance to the herbicide glyphosate, MON 87705 is agronomically and phenotypically equivalent to conventional soybean. Thus the cumulative impacts due to agricultural management and processing practices of MON 87705 would be no different than those due to management practices of commercial soybean varieties. Similarly, there are no changes to industrial processing of MON 87705 relative to the processing of commercial soybean, including specialty soybean.

P.D.7. Potential Impacts to Plant, Animal and Microbial Communities Including Threatened or Endangered Species and Biodiversity

The following section addresses potential impacts due to deregulation of MON 87705 to plant and animal communities, including soil organisms. An overview of the potential impact of glyphosate in the environment may be found in Appendix O.

In assessing the potential impact to plant and animal communities, the potential for gene movement and introgression from MON 87705 was evaluated because movement and establishment of the gene and trait to related species could have indirect impacts to plant and animal communities that extend beyond the original recipient organism. 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 87705 into other species in the U.S. is essentially zero (Stewart et al., 2003); thus, the potential impact of introgression of MON 87707 to sexually compatible relatives on plant and animal communities is nonexistent if USDA-APHIS were to grant the petition for nonregulated status. Additional discussion of the potential environmental impact due to gene movement may be found in Sections X.D.

Animals

Soybean production systems in agriculture are host to many animal species. Mammals and birds may seasonally consume grain, and invertebrates can feed on the plant during the entire growing season. Animals that feed primarily on soybean are seed-feeding insects and rodents found in agricultural fields. Rodents, such as mice or squirrels, may seasonally feed exclusively on soybean seeds. Thus, these animals may have a diet containing significant amounts of soybean seeds. Deer may also browse in soybean fields on the forage and on seed left after harvest.

Agronomic practices used to produce MON 87705 will be the same as those used to produce other glyphosate-tolerant soybean, including Roundup Ready soybean, that were planted on 92% of U.S. soybean acreage in 2008 (USDA-NASS, 2008) so any switch by growers from their current glyphosate-tolerant soybean to MON 87705 would not result in significant impacts on wildlife compared to current soybean production.

Potential impacts to animals would be primarily based on the effects of the introduced protein CP4 EPSPS and the suppression of endogenous *FATB* and *FAD2* RNAs resulting in the improved fatty acid profile of the MON 87705 soybean oil. As discussed previously, there is no meaningful risk to animal or human health from dietary exposure to CP4 EPSPS or the improved fatty acid profile from MON 87705 soybean oil. There are no toxic properties associated with either the CP4 EPSPS protein or with the improved fatty acid profile produced by MON 87705. Furthermore, the composition of the seed, forage and meal produced by MON 87705 is unchanged from conventional soybean meal. This information indicates that there would be no negative effects to mammals that forage on MON 87705. Similarly, it is expected that there would be no impact to birds or other animals that may consume soybean forage or soybean seed. During field trials no changes in insect feeding damage were observed (see Section VIII) indicating similar insect susceptibility for MON 87705 compared to conventional soybean. As MON 87705 exhibits no toxic effects on animals or pollinators of other plants in or around fields cultivated with MON 87705, they will not be affected.

Ninety-two percent of soybean acreage in the U.S. is herbicide-tolerant, with most of these acres planted with Roundup Ready soybean (USDA-NASS, 2008). Consequently, glyphosate is the most widely used herbicide in soybean production. A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development, and does not bioaccumulate in mammals (Williams et al., 2000; Appendix O). An ecotoxicological risk assessment concluded that the use of glyphosate does not pose an unreasonable risk of adverse effects to non-target species, such as birds and fish, when used according to label directions, nor does it pose an unreasonable risk of adverse effects to insects outside of the application area. On the basis of this analysis, deregulation of MON 87705 will not result in significant impacts on animals, including insects that live near or in soybean fields containing MON 87705.

Plants

Soybean production systems in agriculture are host to many plant species. Likewise, the environment surrounding a soybean field varies in plant composition depending on the region. In certain areas, soybean fields may be bordered by other soybean, corn or other crops; fields may also be surrounded by wooded and/or pasture/grassland areas, as well as aquatic environments. Therefore, the types of vegetation, including weeds, around a soybean field depend on the area where the soybean is planted. A variety of weeds dwell in and around soybean fields; those species will also vary depending on the region where the soybean is planted.

If MON 87705 is granted nonregulated status, agricultural practices that are used for biotechnology-derived herbicide-tolerant commercial soybean grown on the majority of U.S. acreage would be used for plant management during the cultivation of MON 87705. MON 87705 does not exhibit characteristics associated with weedy growth and will not compete with plants found outside of agricultural production. Weeds within fields of MON 87705 will be managed using mechanical, cultural, and chemical control measures, as weeds are now managed in commercial soybean systems. Monsanto's glyphosate label provides information regarding appropriate conditions for application of Roundup herbicide agricultural herbicides that are designed to minimize damage to adjacent vegetation. Therefore, the presence of the herbicide- tolerance trait in MON 87705 and the improved fatty acid profile trait is not expected to have a significant impact on surrounding plant communities.

Threatened and Endangered Species

No significant impact to threatened and endangered species is expected from the introduction of MON 87705. Monsanto has considered the potential impact of MON 87705 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 87705, as well as typical agricultural practices associated with cultivation of soybean. As previously noted, consumption of the CP4 EPSPS protein has no toxicity in laboratory testing with mice (see Section X.B.1.). MON 87705 does not express any additional proteins or natural toxicants that are known to directly or indirectly affect a listed TES or species proposed for listing by the U.S. Fish and Wildlife Service. Additionally, MON 87705 contains only those fatty acids that are presently found in soybean oil. MON 87705 has a fatty acid profile that is comparable to commercial high oleic vegetable oils (e.g., high oleic canola, high oleic safflower, high oleic sunflower), that are currently grown inthe U.S.

MON 87705 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, (*Sciurus niger cinereus*), found in areas of the mid-Atlantic Eastern seaboard[2]. It is known to utilize certain agricultural lands readily, and 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 of the CP4 EPSPS protein and MON 87705 on mice and other non-target organisms, respectively, it is concluded that MON 87705 will not have an effect on the Delmarva Peninsula Fox Squirrel.

No impact to any threatened or endangered plant species is expected from the cultivation of MON 87705. Like other *G. max*, MON 87705 will likely be a poor competitor with native vegetation, has no sexually-compatible relatives in the U.S. and will not survive

[2] http://ecos.fws.gov/tess_public/SpeciesReport.do; [Accessed May 14, 2009].

outside of cultivation. Thus, there is no opportunity for MON 87705 to interbreed with any plant species or displace natural vegetation in the U.S.

In a TES risk assessment previously provided to USDA-APHIS in support of Roundup Ready alfalfa petition 04-110-01p Monsanto identified some plant but no animal species that may be at risk from the use of glyphosate-based herbicides in the Roundup Ready crop system this assessment is summarized in Appendix O. To mitigate these potential risks, Monsanto developed Pre-Serve (www.pre-serve.org), a web-based program designed to protect TES plant species from potential impacts resulting from the agricultural use of herbicides that contain glyphosate. Pre-Serve instructs growers to observe specific precautions when spraying glyphosate herbicides on Roundup Ready crops near TES plant species that may be at risk.

Soil Microorganism

No adverse effects on soil microorganisms are associated with MON 87705 nor do the characteristics of the CP4 EPSPS protein or the improved fatty acid profile pose any concern to soil microorganisms. Monsanto presented data in this petition (see Section VIII) demonstrating the lack of impact to symbiotic microbes associated with soybean plants. The *B. japonicum*-soybean symbiosis of MON 87705 was not changed as a result of the introduction of the CP4 EPSPS protein and the improved fatty acid profile compared to a conventional soybean control. MON 87705 contains only those fatty acids that are presently found in soybean oil, and has a fatty acid profile that is comparable to other commercial high oleic vegetable oils. The fatty acids found in MON 87705 have a long history of safe use in human and animal consumption, are naturally present in the environment, and will be broken down and utilized by soil microorganisms in a manner similar to fatty acids found in other food crops.

On the basis of these observations and in conjunction with related phenotypic measurements for MON 87705, no impact on soil microorganisms or soil arthropods is expected from deregulation of MON 87705.

Biodiversity

Analysis of available information indicates that MON 87705 exhibits no traits that would cause increased weediness, that its unconfined cultivation would not lead to increased weediness of other sexually compatible relatives (of which there are none in the United States), and it is unlikely to have effects on non-target organisms common to agricultural ecosystems or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. The use of herbicides in agricultural fields is likely to indirectly impact biodiversity by decreasing weed species present in the field. Agricultural fields are purposefully managed to be weed-free resulting in greater economic benefit to the grower. Ninety-six percent of soybean acreage in the U.S. was treated with glyphosate in 2006 (USDA-ERS, 2007). Therefore introduction of MON 87705 is unlikely to affect the animal or plant communities found in commercial soybean production systems due to both the lack of toxicity of either the CP4 EPSPS protein or the improved fatty acid profile. Because MON 87705 will likely replace soybean varieties that already have

glyphosate tolerance, no significant impacts from its introduction are anticipated. For an overview of cumulative impacts from deregulation of glyphosate-tolerant crops see Appendix N. Based on this analysis, it is concluded that deregulation of soybean varieities containing MON 87705 would have no significant impact on biodiversity.

No impacts to plant and animal communities or threatened and endangered species have been identified for MON 87705. No cumulative impacts to plant and animal communities or threatened and endangered species have been identified.

P.D.8. Other Cumulative Effects

Conventional Breeding with Other Biotechnology-derived or Conventional Soybean Varieties

As previously mentioned, several biotechnology-derived soybean crop varieties have been deregulated or are under consideration for deregulation, and a list of the events codes deregulated by USDA is presented in Table P-5 below. MON 87705 may be bred with these deregulated biotechnology-derived soybean crop products as well as with conventional soybean, creating new improved varieties. APHIS has determined that none of the biotechnology-derived individual soybean products it has deregulated display increased plant pest characteristics and that any progeny derived from crosses of these soybean crop products with other conventional or biotechnology-derived soybean are unlikely to exhibit new plant pest properties.

An assessment of the stability of the genetic insert in MON 87705 was conducted, and data have been presented in this petition demonstrating that MON 87705 is stable in progeny. Having established that the genetic material is stable and that MON 87705 is inherited in a Mendelian fashion, and based on experience with MON 87705 in Monsanto's plant breeding program, it can be concluded that the phenotype of MON 87705 is likewise stable. Conventional breeding has an established history of safe use, and use of MON 87705 in breeding programs is expected to behave in a manner similar to other conventional traits and biotechnology-derived traits. For example, in an assessment by McCann et al. (2005), it was shown that during three years of breeding into multiple varieties, the composition of glyphosate-tolerant soybean remained equivalent to that of conventional soybean. Given that there have been no plant pest characteristics associated with MON 87705, or with any of the previously deregulated events listed below, no significant impacts are expected to other soybean varieties through the use of MON 87705 in breeding programs and in combination with any of the previously deregulated soybean crop products.

All biotechnology-derived soybean products on the market today have satisfactorily completed the FDA consultation process established to review the safety of foods and feeds derived from biotechnology-derived crops for human and animal consumption (see Table P-5). As mentioned above, breeding with modified oil soybean products on this list, while not currently envisioned, would predictably alter the fatty acid composition of MON 87705 (i.e., modify the oil profile to a combination of the individual trait

characteristics). No impacts to public health (e.g., food or feed safety) are expected due to combination of these events through conventional breeding because the deregulated events have a history of safe use and on the basis of knowledge of the type of modifications made to each of the deregulated events, the biochemical pathways are not likely to unexpectedly interact or result in the production of novel constituents.

The decision to deregulate MON 87705 would also allow for breeding of this product with conventional soybean varieties of diverse genetic backgrounds. These varieties will include commercial varieties with low linolenic acid levels which can further enhance the oxidative stability of the soybean oil. In addition, MON 87705 will be combined using traditional breeding methods with other biotechnology-derived traits, including glyphosate tolerant MON 89788. This combined trait product will incorporate the improved fatty acid profile of MON 87705 with the superior germplasm and 4-7% yield advantage of MON 89788 to deliver the best agronomic platform to farmers. No impacts to public health (e.g., food or feed safety) or environmental safety are expected due to the breeding of MON 87705 with these other soybean varieties because these varieties have an established history of safe use.

Furthermore, the process of conventional breeding to combine biotechnology-derived traits or biotechnology-derived and conventional varieties to produce combined trait products would likely identify and remove off-types during development of new varieties. Breeders use standard testing and assessment procedures to further examine and confirm the equivalence of the combined trait products, compared to the single event products, in terms of phenotypes, agronomic characteristics, and the efficacy of the traits.

Table P-5. Deregulated or Submitted 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

Source: http://www.aphis.usda.gov/brs/not_reg.html

Economic Impacts of Biotechnology-derived Products

In a recent study, economists Brookes and Barfoot (2006) 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. In the U.S. the impact of herbicide-tolerant soybean has primarily been to reduce the cost of production. In the early years of adoption the savings were between $25-34/ha. In more recent years the cost savings have risen to $60-78/ha based on comparison of herbicide regimes that would be required to deliver a comparable level of weed control. It is estimated that the cumulative U.S. farm income benefit from 1996-2006 was approximately $8.73 billion USD. Other soybean production countries (Argentina, Brazil, Canada, South Africa) have also experienced cost savings from the adoption of biotechnology-derived herbicide-tolerant soybean varieties. In addition to cost savings, countries like Romania and Mexico have also experienced yield gains (31% and 9%, respectively) from improvements in weed control.

It is not known how many U.S. acres MON 87705 will be grown on when commercialized. However, MON 87705 may provide benefits to U.S. soybean producers similar to herbicide-tolerant biotechnology-derived soybean commercialized on broad acres across the U.S., and provide growers with another high-value specialty soybean product option.

P.E. Highly Uncertain, Unique or Unknown Risks

MON 87705 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 thirteen biotechnology-derived soybean crop products. MON 87705 offers a low saturated fat, high oleic soybean oil alternative that has significantly improved heat and oxidative stability. Introduction of previous biotechnology-derived crops have resulted in no unexpected effects on the quality of the human environment as defined under NEPA and have provided benefits to growers, consumers and the environment. In this respect, a decision to deregulate a new biotechnology-derived soybean product is not precedent setting nor are the effects to the quality of the human environment highly uncertain or unpredictable.

P.F. Summary

MON 87705 has been thoroughly characterized and the extensive body of information presented in Sections I through X of this petition demonstrates that MON 87705 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 specialty soybean crop products have benefited farm income in the U.S. The amount of land devoted to farming (specifically to corn or soybean) has not changed with the introduction of biotechnology-derived crops. Similarly, no significant 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 87705. MON 87705 will utilize common cultivation practices typically employed for production of commodity soybean and many types of specialty soybean, and management practices typically used for

specialty soybean (i.e., IDP production practices). Hence, agricultural practices would not be impacted if MON 87705 were deregulated.

The opportunity for growers to produce soybean with a low saturated fat, high oleic acid fatty acid profile that has significantly improved heat and oxidative stability relative to conventional soybean is a positive benefit from an economic and human health perspective. Additionally, MON 87705 soybean and its fractions have, other than the intended changes to fatty acid composition, been demonstrated to be equivalent to conventional soybean. Therefore, MON 87705 soybean and its fractions are as safe and as wholesome for food and feed purposes as conventional soybean. For these reasons, the proposed action to grant nonregulated status to MON 87705 does not represent a significant impact to the environment.

Appendix P References

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Petition for the Determination of Nonregulated Status for Improved Fatty Acid Profile MON 87705 Soybean

ADDENDENDUM I

Market Impact of MON 87705 High Oleic Soybean Oil

Submitted April 7, 2011

OECD Unique Identifier: MON-87705-6
Monsanto Petition Number: 09-SY-201U

Introduction

Overall total use of oils derived from plant sources (vegetable oil) for food and industrial applications exceeded 14 million tons in North America in 2009. Due to its abundance, reliable supply and price, soybean oil is one of the most highly used of the vegetable oils (Attachment I, Table 3.1, pg 62). In 2009, soybean oil accounted for approximately 8.2 million tons of food and 1.2 million ton of industrial vegetable oil uses (See Attachment I, Part 1b, pg 17-18). Soybean oil use in foods peaked at roughly 9.3 million tons in the 2006/2007 timeframe just before the U.S. Food and Drug Administration required food labels to report *trans-fat* content in 2006. (http://www.fda.gov/Food/LabelingNutrition/ConsumerInformation). The demand for soybean oil for frying and baking applications has declined in recent years due to the desire to lower unhealthy *trans-fat* in the diet and changes in food labeling policy.

High oleic soybean oil produced using MON 87705 has a fatty acid profile that has reduced saturated fats, and higher monounsaturated fat. These properties, particularly the reduction in polyunsaturated fats (18:2), result in increased oil oxidative stability greatly improving oil performance in frying and baking applications. The decrease in saturated fat (16:0, 18:0) further improves the nutritional profile of the oil for use in food preparation. MON 87705 will be bred into Vistive® low-linolenic soybean further improving the oil profile by lowering the level of linolenic acid and eliminating the need to hydrogenate the oil.

Monsanto has conducted a market and trade assessment to evaluate the potential impacts on commodity soybean oil and other vegetable oils due to the introduction of high oleic soybean oil. The assessment considered that other vegetable oils including high oleic vegetable oils are currently on the market and are routinely handled by oil processors and food formulators. The system responsible for production, harvest, processing and handling of soybean and other vegetable oils includes critical control points as well as economic incentives for identity preserved soybean oils and other vegetable oils. Typically, the processor will assess the fatty acid profile of the oil sold to a food manufacturer prior to sale. If the fatty acid profile were different from commodity soybean oil, the oil could be blended (a common industry practice) to reach the appropriate fatty acid content based on specific food applications. On the basis of this assessment it is concluded that the introduction of high oleic soybean oil will have little if any potential for negative market impacts. In the event that comingling did occur, the economic impact of comingling to a food manufacturer would be minimal and remedied through blending.

A summary of the intended uses for high oleic soybean oil, current processes for handling vegetable oils and potential impacts to commodity soybean oil as well as other vegetable oils is provided in this document.

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Intended Uses of High Oleic Soybean Oil

Commodity soybean oil does not have the optimum fatty acid profile to satisfy many of the needs of the food industry[1]. The fatty acid profile for soybean oil does not provide optimal oxidative stability compared to other vegetable oils unless modified via hydrogenation or through blending with other vegetable oils. Hydrogenation creates unhealthy *trans-fat* making soybean oil less desirable for use in some applications. In addition, due to low oxidative stability, commodity soybean oil is not highly stable for cooking and frying when used in these applications, causing several operational challenges such as more frequent exchange of vegetable oil stocks and/or replacement of oil and polymerization, leading to frequent costly equipment cleaning. For these reasons, in recent years, soybean oil has lost share to other vegetable oils in food applications (Wilson, 2004; Attachment I, Part 2, pg 38).

Given recent food labeling policy changes and modifications to dietary guidelines, the food industry is seeking vegetable oils that not only have enhanced functionality but minimize levels of trans- and saturated fat; the balance of fatty acids (high oleic, low saturated fat, low linolenic) is often difficult to achieve with current vegetable oil options. Soybean oil competes with other oils such as palm, canola, sunflower, peanut and corn. These oils are generally blended to satisfy food companies seeking to address functional or nutrition related needs. High oleic soybean oil can provide a complementary option either as a straight replacement of blended oils or as a blending alternative to end users. While blending achieves the food company's objective, it is costly due to increased handling, average oil price and required technical support (Attachment I, Part 4, pg 75).

Through conventional plant breeding, MON 87705 will be bred with Vistive low-linolenic soybean. Vistive low-linolenic soybean was developed through conventional plant breeding to produce lower levels of linolenic acid allowing food companies to avoid hydrogenation of soybean oil helping to eliminate unhealthy *trans* fatty acids from the diet. The low linolenic soybean has a mutation in the *Fad3-1c* and *Fad3-1b* genes that result in reduced levels of linolenic acid. The Vistive low-linolenic soybean oil is currently commercially sold in the United States. The soybean oil from MON 87705 in the Vistive low-linolenic soybean background has the following specification: palmitic acid (< 4.0%); stearic acid (< 4.0%); oleic acid (55 - 85%); linoleic acid (8 – 30%); and linolenic acid (< 4.0%). The modification of the fatty acid profile provides an equal or better alternative to vegetable oil options available today and offers the opportunity to reduce the practice of blending which has become the solution for fry applications to avoid *trans-fats*. The observed fatty acid profile of MON 87705 soybean and MON 87705 in a Vistive low-linolenic soybean genetic background are presented in Table 1.

[1]http://qualisoy.com

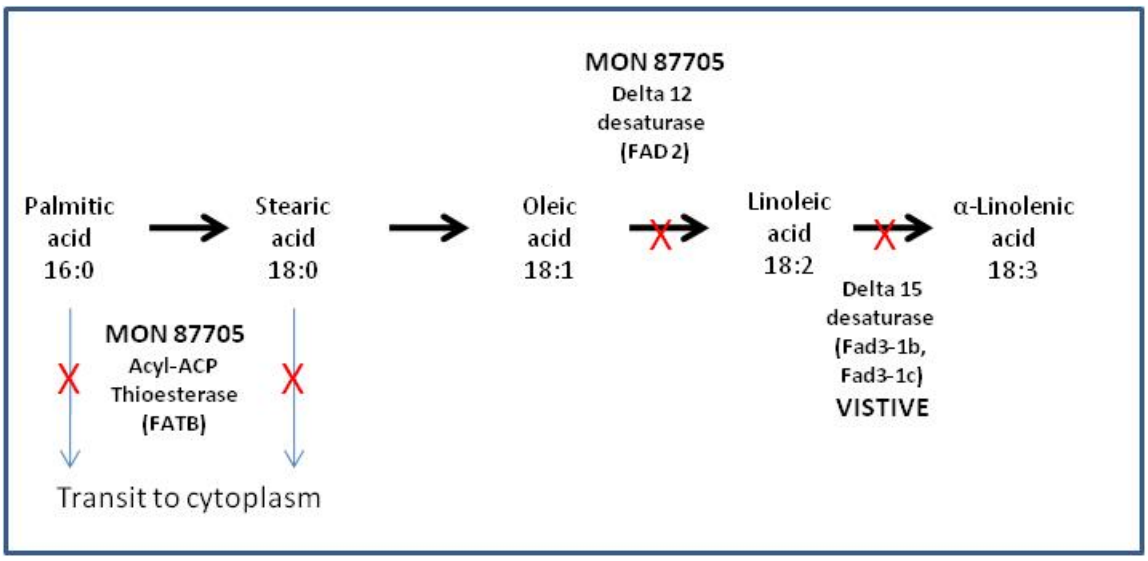


Figure 1. Fatty acid synthesis in MON 87705 in a Vistive low-linolenic genetic background

Table 1. Fatty acid composition (% weight of soybeans) of MON 87705 soybeans and MON 87705 in a Vistive low-linolenic soybean background

Year	C16:0	C18:0	C18:1	C18:2	C18:3
MON 87705					
2007[1]	2.36	3.31	76.47	10.10	6.69
MON 87705 in a Vistive low-linolenic soybean background					
2007[2]	2.60	3.05	77.27	13.77	2.63
2008[2]	2.47	2.97	75.30	15.60	2.96
2009[3]	2.31	3.29	71.21	19.03	3.20
2010[4]	2.20	3.43	76.83	13.73	2.47

[1]Samples were obtained from field trials conducted in Chile in the 2007/2008 growing season. See USDA Petition Number 09-201-01p.
[2]Grown in Jerseyville, IL.
[3]Samples were obtained from 8 U.S. locations (2 sites each in the states of IA, IL, IN, and OH). Value is mean of the 8 individual site values.
[4]Samples were obtained from 3 U.S. locations (2 sites in IL and one site in OH). Value is mean of the 3 individual site values.

The safety of MON 87705 soybeans in a Vistive low-linolenic soybean genetic background is based on the long-standing history of safe consumption of the levels and types of fatty acids contained in commercial vegetable oils with a fatty acid profile similar to MON 87705 soybeans

in Vistive low-linolenic background. Monsanto has completed a safety assessment[2] of high oleic soybean oil and high oleic soybean oil is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use. MON 87705 soybean in a Vistive low-linolenic soybean genetic background does not contain any new fatty acids that are not presently found in conventional food oils and the fatty acid profile of this oil is similar to many other commercial oils currently available including canola, olive oil, high oleic safflower oil, and high oleic sunflower oil (See Table 2). The oil from MON 87705 soybeans in a Vistive low linolenic soybean genetic background provides options for food formulation that is virtually *trans* fat-free, and, unlike palm oil, is low in saturated fat.

Table 2. Fatty acid composition (% weight) of MON 87705 in Vistive background in comparison to soybean oil and other high oleic vegetable oils [1]

Oil	Saturated fats	*trans* fats	Oleic acid	Linoleic acid	Linolenic acid	Other fatty acids
Soybean oil, all purpose	15.2	0.7	22.6	50.1	6.5	4.9
PH Soybean [2]	24.7	34.1	31.4	4.5	0.2	5.1
MON 87705 in Vistive background [3]	6.8	0.22	71.7	16.9	2.9	1.5
HOSO (305423) [4]	14.27	0	70.6	5.5	7.2	3.4
Olive oil	13.8	0	71.3	9.8	0.8	4.3
HOLLCO [5]	3	0	84	7	2	4
High oleic canola	6.5	0.8	70	14.3	2.6	5.8
Canola	7.6	1.6	60.6	17.7	6.4	6.1
High oleic Sunflower	9.7	0	82.6	3.6	0.2	3.9

[1] The fatty acid profiles of PH soybean oil (partially hydrogenated soybean oil), olive oil, canola oil, high oleic canola oil, and high oleic sunflower oil were obtained from the USDA nutrient database (USDA 2008, National Nutrient Database for Standard Reference. U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Nutrient Data Laboratory; Beltsville, Maryland. Available from: http://www.nal.usda.gov/fnic/foodcomp/search/).
[2] PH Soybean: partially hydrogenated soybean oil.
[3] MON 87705 in Vistive background fatty acid composition is based on the pooled analysis of 8 oil lots.
[4] HOSO (305423): high oleic soybean oil from Pioneer/DuPont, defined in Delaney (2008).
[5] HOLLCO: High oleic low linolenic canola oil, values from Möllers (2004).

[2]See FDA GRAS assessment at:
http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm061846.htm#Q1

Vegetable oils are used in twelve broadly grouped end use sectors: snacks, salad oils (use in mayonnaise, salad dressings and other food products), margarine, bottled oils (home use), par fry, ingredient, confectionary, baked goods, industrial fry, restaurant, institutional, and food service (Attachment I, Part 2, pg 37). High oleic soybean oil is targeted for ten of the twelve end use sectors with bottled and salad oils not considered a commercial target for high oleic soybean oil because commodity soybean oil currently serving these sectors does not require hydrogenation and products currently have acceptable shelf lives.

Industrial uses such as bio lubricants and biodiesel are also possible end uses of high oleic soybean oil. High oleic soybean oil is a renewable resource and is an attractive option as the price of crude petroleum continues to increase. The increased levels of oleic acid in high oleic soybean oil enhance the stability and lubricity of the oil making it a superior bio lubricant compared to petroleum based oils and a good feedstock for biodiesel (Graef et. al, 2009).

Food and industrial end use sectors are currently supplied by commodity soybean oil as well as domestic and imported blends of other vegetable oils (e.g. palm oil). Thus, high oleic soybean oil would potentially replace commodity soybean oil and oil blends currently used for these applications.

Market Potential of High Oleic Soybean Oil

In order to assess the market potential for high oleic soybean oil, Monsanto commissioned a study that included an in-depth analysis of the vegetable oil market in North America that was performed by LMC International (Attachment I). The study was conducted to assess the potential demand for vegetable oil with the fatty acid properties of high oleic soybean oil. Factors considered included current uses of vegetable oils and their physical properties, health attributes of the oil, availability and price. The market potential estimate assumed that high oleic soybean oil was used in the target food sectors described above as a direct replacement for hydrogenated soybean oil and other vegetable oils (such as canola, corn, palm and sunflower) used in food applications (Attachment I, part 5; pg 88). The demand for high oleic soybean oil is price sensitive relative to other vegetable oils. To estimate of the maximum high oleic soybean the lowest price premium ($50/metric ton) was used. At the lowest price premium, high oleic soybean would achieve a demand for the oil estimated at 3.5 million metric tons. On the basis of this analysis, approximately 16 million acres of high oleic soybean would satisfy the demand if high oleic soybean oil were to replace current uses of hydrogenated soybean and other high oleic vegetable oils in food and industrial applications (Attachment I, Part 5, pg 90)[3]. Additional acres would likely be planted to supply high oleic soybean oil for industrial uses. Based on assumptions regarding product adoption and acceptance by the industry, Monsanto estimates it would take at least a decade for high oleic soybeans to achieve this acreage and at peak

[3] One bushel of soybean = 60 lbs and contains 10.7 lb oil (Wilson, 2004); average yield of soybean = 43.7 bushels per acre in 2010 (USDA NASS: http://www.nass.usda.gov)

penetration, this level would represent approximately 23% of total planted soybean acres[4]. Subsequently, there would continue to be adequate supply of both commodity and high oleic soybeans to serve the vegetable oil market needs.

Production and Handling of Vegetable Oils

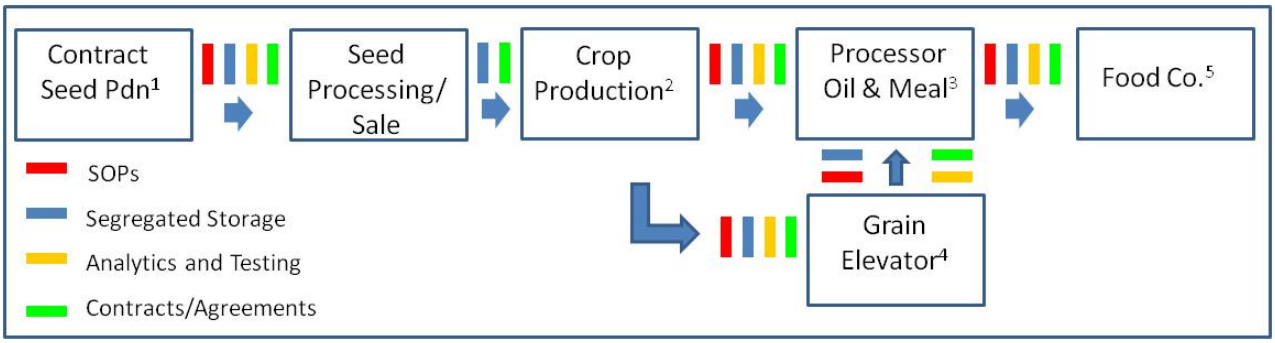
Crop production and distribution systems as well as oil processors and food manufactures have demonstrated the ability to handle multiple vegetable oils derived from numerous crop species. A summary of the current practices including critical control points for production of soybean oil and specialty soybean oil (e.g. Vistive low linolenic) is provided below. There are numerous vegetable oils on the market today including: palm, coconut, palm kernel, cottonseed, soybean, groundnut, rapeseed and sunflower (Attachment I, Part 2, pg 38). Other minor use oils include olive, corn, high oleic canola, high oleic sunflower oil and low linoleic soybean oil (Vistive). The processing industry (e.g. Cargill, Bunge, ADM, etc.) and end users of such oils are highly adept at handling these vegetable oils in their normal course of business. As a result, processors have developed methods to segregate multiple vegetable oil feedstocks as they are financially motivated to prevent mishaps with their customers. High oleic soybean, for planting and subsequent processing, would be introduced to the market place as an option for processors to supply food companies and those who seek a U.S. based vegetable oil option.

Once key global market approvals are obtained, oil from MON 87705 will be available as an option for use by food processors and will be produced in an identity preserved (IDP) fashion similar to the system that has been successfully implemented for production of Vistive low linolenic soybean and other specialty vegetable oils. IDP practices are implementd for value added specialty soybean to capture the enhanced value of the product and ensure that the end-user or processor receives the soybean with the desired identity, fatty acid profile and quality. Vistive low-linolenic soybean was made available to growers in 2005 and has been grown on over 3.0 million acres since its initial launch.

A summary of the quality control systems associated with seed production, crop production, processing and end uses of high oleic soybean oil is presented in Figure 2.

[4] Assumes 70 million acres of soybean are planted.

Figure 2. Production and use checkpoints for high oleic soybean



[1]Monsanto contracts soybean seed production with growers under ISO standards.
[2]Growers produce crop under a contract – typically growers have segregated on farm storage of harvested grain. Grower may deliver soybean directly to a processor or to a grain elevator.
[3]Processor has on-site segregated storage, performs testing of soybean with on-site NIR method. The fatty acid profile of the ingredient is analyzed prior to acceptance of soybean and on processed oil. Test results are provided to the Food Co. by the processor.
[4]Grower delivers to elevator, analytical testing of soybean using on-site NIR method, elevator holds soybeans until called by processor. Delivery of grain to processor triggers same process as delivery from grower.
[5]Food Co. handles multiple vegetable oil ingredients. Food company follows existing ingredient and batch identification procedures, and product tracking throughout the production and distribution system including retailer shipments.

Seed Production and Processing. Monsanto is a leader in crop biotechnology having successfully introduced numerous biotech crops to the marketplace. Monsanto has developed and implemented seed quality practices to assure that soybean seed meets the standards established for purity of a trait. These standards apply to all soybean seed sold by Monsanto and are based upon measures that seed producers put in place to assure the genetic purity of improved planting seed. This system is used to assure that farmers receive seed of known quality with a minimum level of off types.

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

Commercially certified soybean seed must meet state and federal seed standards and labeling requirements. The Association of Official Seed Certifying Agencies (AOSCA) standards for certified soybean seed are as follows: 98% pure seed (minimum), 2% inert matter (maximum),

0.05% weed seed (maximum, not to exceed 10 per lb.), 0.60% total of other crop seeds (maximum), 0.5% other varieties (maximum, includes off-colored beans and off-type seeds), 0.10% other crop seeds (maximum, not to exceed three per lb.), and 80% germination and hard seed (minimum) (AOSCA, 2009). Seed that meets or exceeds these standards are provided in appropriately labeled seed bags to growers

Production of Soybean Grain Containing High Oleic Soybean Oil. Specialty soybean (e.g. Vistive low linolenic) is currently produced under contracts issued by processors or elevators. It is expected that high oleic soybean will be produced using this system as well.

There are three major factors needing to be satisfied before a farmer will produce a specialty soybean containing a modified soybean oil, like high oleic. These factors have become evident in Monsanto's experience in the crop production of Vistive low linolenic soybeans.

1. Yield of the soybean must be comparable to commodity soybeans routinely planted
2. The option to market, price & deliver the grain must be comparable to commodity grain
3. The premium paid to the farmer to offset the cost to identity preserve the high oleic containing grain must provide incremental income opportunity above the production of commodity grain. The income opportunity cannot be realized by the farmer unless the grain is delivered to the processor within specifications or in its identity preserved state. Therefore, the motivation is a financial incentive for the farmer to avoid comingling with commodity grain, keeping all the grain identity preserved within this closed loop system of seed – farmer - processor. Many farmers willingly choose to plant all their acreage to this specialty soybean because it eliminates any risk of contamination from inadvertent errors that may occur during the harvest. If a farmer chooses to produce the specialty grain for the processor, the farmer will arrange to store the grain on farm or at their local elevator, if the elevator is participating in the specialty program with the processor.

The steps involved in securing grain production are:

1. Monsanto will sell and distribute high oleic soybean seed to the farmer after the farmer has signed and agreed to the conditions of Monsanto's Technology and Stewardship Agreement. The agreement will mandate that the farmer sell any soybean produced into an identity preservation channel (See Figure 2).
2. Processors will be responsible to contract with elevators and growers the acreage needed to fulfill the expected demand for high oleic soybean.
3. After harvest, the farmer will deliver the grain produced to the location specified in his contract, either a participating elevator or processor. If to the elevator, the elevator will keep the grain segregated from commodity grain and pay the farmer his premium, provided it passes the analytical testing. The elevator will deliver the grain to the processor as delivery windows and crush schedules have been established. Upon delivery of grain by the farmer, samples will be analyzed from every truckload by Near Infrared Transmittance (NIT) developed by Monsanto. This will confirm the grain contains high oleic soybean oil as required by the production contract. Upon confirmation of grain that meets specification of high oleic soybean grain, the processor or elevator will approve the premium payment to the farmer.

In the event that high oleic soybean do not meet the minimum specifications established for oil quality, the soybean would be isolated from other high oleic or commodity soybean and blended with an appropriate quantity of high oleic soybean to meet the specifications required for a food application. Monsanto has been conducting field trials with high oleic soybean since 2005 and has considerable experience with trait performance in various genetic backgrounds and under various climatic conditions. Trait performance has been consistent over several years of breeding (See Table 10). All soybean varieties that Monsanto will introduce must meet specifications for agronomic performance and oil quality. While it is not envisioned that any of the high oleic soybean varieties will underperform, blending with other high oleic soybean is the most likely remedy.

Grain Elevator. Grain elevators play an important role in specialty programs with their long term storage of the grain. Since processing facilities crush soybeans throughout the calendar year, soybeans used to supply these crush plants need to be stored year round. Farmers typically prefer to empty their storage prior to planting of the new crop and prior to temperature warm up in the spring. The warm spring and summer weather present challenges as condensation can build up in the bins creating moisture related issues and grain eating insects become more active. Farmers prefer to avoid these high management grain conditions. Commercial elevators have expert grain managers on staff to monitor grain quality and keep grain in condition in all weather situations presented throughout the year.

Commercial grain elevators are also better equipped to ship grain to processors during times of severe weather or when farmers cannot get to their bins when roads are impassable.

Processors will therefore enter into supply contracts with commercial grain elevators for these reasons. Contracts will be “acre” based, established to fill bin capacity that has been agreed upon by both parties. Grain elevators will then contract with farmers directly, providing harvest storage terms to the farmer with premiums to be paid that have been agreed upon with the processor. The elevator pays the farmer the specialty grain premium upon successful analytical testing by NIT performed at the elevator location. NIT testing equipment will be provided by Monsanto to participating elevators identical to the equipment provided to processors.

In order for the elevator to be reimbursed for the premiums paid out to farmers that have delivered to them, the elevator must in turn preserve the identity of the grain as it is delivered to the processor.

Every load delivered to the processor by the elevator will be analyzed using NIT technology. Processors will approve the premium payment to the elevator after analysis of the grain confirms high oleic soybean oil.

Processing Oil and Meal. Specialty high oleic soybean grain will be introduced into the processing plant as it is running commodity soybean grain. Upon the transition from commodity soybeans in the plant, the processor will ensure that the equipment is lined out and operating within normal and acceptable limits and parameters using commodity soybean. High oleic soybeans will then be moved through the plant continuously until all specialty grain located at

the plant is gone. After all of the specialty high oleic soybeans have moved through the seed prep building, the bins and conveyors feeding the seed prep building will be filled with commodity soybeans to keep the plant running continuously. Commodity soybeans will then be processed as normal.

Upon the transition from commodity soybeans to the high oleic soybeans, a sample of crude oil is required to confirm the presence of the appropriate fatty acid composition (FAC) unique to high oleic oil. Until the fatty acid composition of the crude oil exiting the extractor is of the appropriate composition, as determined by the plant manager, off spec crude oil will be sent to a tank that will be designated for flush oil. This will contain all oil before and after the collection of "on-spec" high oleic oil. High oleic crude oil that is collected with the appropriate FAC will be designated for further refining, bleaching, and deodorization. The processor's lab will be used to aid in identifying the appropriate time to start collecting crude high oleic oil exiting the extractor that will undergo further refining. The lab will use gas chromatography equipment to analyze the crude oil to determine when on spec high oleic oil can be collected from the extractor. A tank approved for high oleic oil will be assigned by a processor employee. After all high oleic grain has been processed, the seed extraction area will be flushed with commodity soybeans, and regular commodity oil will be produced. During the flush, crude oil exiting the extractor will be sampled and analyzed to confirm the equipment has been flushed to an acceptable level, yielding commodity soybean oil.

Processes are established by the processor to ensure the transfer of high oleic oil to the crude oil tank farm to minimize loss and maximize quality. The lab at the processor will utilize appropriate analytical methods to determine when the high oleic oil is of proper quality to send to the main crude tank(s) that will supply oil for further refining. Commodity soybean flush oil prior to and after the crush of the high oleic oil will be collected in tanks designated as flush, and will be blended with commodity soybean oil to bring the flush oil into a specification range necessary to be sold as commodity soybean oil.

Food Company. Oil will be supplied to the food company by oil processors and suppliers. The processor or supplier will test the oil and assure that it meets specific customer requirements including quality factors (e.g. peroxide value, oxidative stability index, color, flavor) and oil composition (customized blends, specified fatty acid composition). Foodservice distributors typically obtain the oil from the oil processors and deliver it to each foodservice outlet (individual stores, caterers, cafeterias, etc). From the time the oil is packaged, until it is utilized at the specific customer's facility, there is proper identification of the oil through labeling and manufacturing codes allowing for sufficient product traceability if needed. Individual facilities will utilize proprietary inventory and ordering systems that are in place to insure that the appropriate oil is ordered, delivered and utilized.

High oleic soybean oil will be used by the food industry in several ways. One specific type of food company customer will be foodservice operators including quick service restaurants, casual dining and full service restaurants who will use high oleic soybean oil as a fry medium and in

meal preparation. Food companies will also use high oleic soybean oil as a food ingredient in a range of food products including as spray oil on crackers and snacks, or as a component of oil blends used in shortenings and other foods. Each individual food company has in place systems for ingredient (oil) ordering, receipt, storage, access and lot identification at specific manufacturing locations, as well as finished product (the food which incorporates high oleic soybean oil) batch identification, manufacturing facility, storage, shipment to distribution centers, customer order picking, customer order shipment and receipt. Appropriate procedures are currently in place to insure traceability from receipt of the ingredient through distribution to a specific retailer's facility. Food companies routinely conduct mock recalls including use of the media should the need arise. Food manufacturing facilities also comply with federal and state requirements for good manufacturing practices and product traceability. Supply chain consultants can be employed to confirm appropriate systems have been established that meet ingredient and product traceability requirements.

Stewardship of High Oleic Soybean

Monsanto is committed to product stewardship and to implementing BIO's "Excellence through Stewardship" program and Product Launch Stewardship Policy[5]. Monsanto considered Annex 2 "Special Use traits in Commodity Crops" [6] to develop launch plans for high oleic soybean oil including: (1) identifying relevant stakeholders for the trait and crop and engaging them in dialogue regarding use of high oleic soybean oil and potential impacts to vegetable oil markets, (2) conducting a market and trade assessment, including securing regulatory approvals in key export countries prior to full commercial launch, (3) developing a risk mitigation plan, and (4) undertaking appropriate outreach, necessary to educate stakeholders and implement the management plan for high oleic soybean oil. These actions protect against adverse impacts to trade of soybean due to the introduction of a new biotechnology improved soybean.

Stakeholder Dialogue

Monsanto is committed to dialogue with key industry stakeholder groups and has held several meetings with the National Oil Processors Association (NOPA) as well as other key industry associations such as the North American Export Grain Association (NAEGA), National Grain and Feed Association (NGFA), North American Milling Association (NAMA), American Bakers Association (ABA), and Grocery Manufacturers Association (GMA). Soybean grower organizations: American Soybean Association (ASA), United States Soybean Board (USB), and many state soybean associations. In addition QUALISOY, a collaborative program sponsored by the USB that serves as an independent, third part resource for information on trait-enhanced soybean oils, has been kept informed on the plans for this product, along with leaders in dietary and nutrition fields.

[5] http://www.monsanto.com/ourcommitments/Pages/product-stewardship.aspx
[6] http://www.bio.org/letters/Product_Launch_Stewarship_12_10_09.pdf;

Market and Trade Assessment

Monsanto has conducted a market and trade assessment to determine the impact of the introduction of high oleic soybean oil. Soybean is a globally traded commodity with the U.S. being the top global producer (Soyatech, 2010). Biotechnology-derived crops and their use as food and feed are subject to regulation in many countries. In order to support continued trade in soybean, Monsanto is pursuing regulatory approval for MON 87705 in all key soybean import countries with a functioning regulatory system to support the flow of international trade. International regulatory authorities are evaluating the biotech component as well as the modified oil fraction. It is expected that uses of high oleic oil soybean will be similar on a global basis.

There would be no impact to human heath or to the use of any of the other processed fractions produced from soybean due to comingling of high oleic soybean with commodity soybean. Monsanto has completed a GRAS assessment of the high oleic soybean oil (MON 87705 in a Vistive background)[7] and has completed the biotechnology consultation on MON 87705 with the U.S. FDA[8]. The GRAS assessment has been evaluated by FDA and the agency had no further questions. The biotechnology consultation considered the food and feed safety impacts due to the genetic modification process, RNAi suppression of two endogenous enzymes resulting in modification to fatty acid metabolism, as well as exposure to the CP4 EPSPS protein. The GRAS assessment considered the nutritional impact of total replacement of commodity soybean oil with high oleic soybean oil. High oleic soybean oil is similar to other high oleic vegetable oils that are commonly consumed producing no harmful effects to humans. Hence, Monsanto as well as a panel of qualified scientific experts have concluded that high oleic soybean oil is generally recognized as safe.

Information provided to USDA showed that the impact due to the suppression of the two endogenous enzymes is restricted to seed. With the exception of the intended changes in fatty acid composition and presence of the CP4 EPSPS protein, the soybean meal and other processed fractions used for animal feed and human food applications are compositionally equivalent to commodity processed soybean fractions[9]. Monsanto provided information to USDA in the petition demonstrating that the composition of the meal was compositionally equivalent to meal derived from conventional soybean and safe and wholesome for food or feed applications.

Monsanto has assessed the impact of the introduction high oleic soybean oil on commodity and other vegetable oils. In response to stakeholder dialogue, Monsanto was specifically requested by NOPA to address the impact of comingling of commodity soybean oil with high oleic soybean oil to bottled oils (100% commodity soybean oil) and severe heat processing (e.g. impact to frying applications and sensory properties of prepared foods). This assessment has been shared with key stakeholders such as NOPA, NAEGA, ASA, Qualisoy, USB and GMA.

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[7]http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm061846.htm#Q1

[8]Biotechnology notice: BNF 121: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=bioListing; GRAS notice: GRN 306 - http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&page=2

[9] USDA Petition Number 09-201-01p, Section VII.C.

Hypothetical impacts were assessed using the following scenario: (1) high oleic soybean grain or oil went unnoticed and was comingled (e.g. harvest mistake, inadvertent mixing of grain during transport, mistaken delivery of grain, etc…), (2) the comingled soybean were delivered to an oil processor and mixed with commodity grain or soybean oil, (3) the oil or grain was not analyzed for oil composition by anyone in the food supply chain, and (4) the comingled oil was used in the food or feed supply chain. This scenario is highly unlikely given existing critical control points embedded in the production handling and processing system and because high oleic soybean oil is expected to be produced in a contractual identity preserved system in order to preserve its premium value. Processors will originate the supply of high oleic soybeans and as they have extensive experience managing, segregating, testing, formulating and blending other sources of high oleic vegetable oils such as high oleic canola and high oleic sunflower oil.

Bottled vegetable oil is not an intended use for high oleic soybean oil. However, bottle oil was used to assess potential impacts at the request of NOPA. It represents a readily measurable impact that could occur if high oleic soybean were comingled with 100% commodity soybean. Mixing high oleic soybean with commodity soybean will impact to the levels of fatty acids in commodity soybean. This effect is measurable and can potentially translate into downstream effects to food labeling, oil quality, and functionality. Even though such comingling is improbable, potential impacts were assessed at several different comingling levels ranging from 4% and up to 30% using four different parameters: (1) food ingredient labeling, (2) nutritional facts panel labeling (3) functionality of the oil, and (4) sensory evaluation.

Food Ingredient Labeling Codex Specification. Effects were assessed on the ability to meet USP Food Chemical Codex (USP FCC) soybean oil labeling specification. In Table 3 the impact of varying amounts of high oleic soybean oil comingled with commodity soybean oil on the ability to meet specifications for major fatty acids is evaluated. The USP FCC is a compendium of internationally recognized standards for the purity and identity of food ingredients and is used as a set of agreed standards between buyers and manufacturers of food ingredients[10]. Based on this analysis the most sensitive fatty acid is 18:2 linoleic acid. If high oleic soybean were co-mingled with commodity soybean oil at levels above 14%, the linoleic acid (C 18:2) content of the resulting oil following processing would fall below the USP FCC specification for commodity soybean oil. At levels above 15% oleic (C 18:1), linoleic acid would be out of the specifications for soybean oil.

To put this into perspective, 15% comingling could occur due to a farmer mistakenly harvesting approximately three acres of high oleic soybean (assuming yield = 43 bushels/acre) and comingling it with commodity soybean in a grain truck hauling the standard load limit with a capacity of 900 bushels. If the grain were delivered to a grain elevator or directly to a processor, it would be mixed with other commodity soybean grain thereby diluting the high oleic soybean considerably with commodity soybean. The capacity of grain elevators or bin storage tanks is highly variable; however, bin capacities up to and

[10] http://www.usp.org/fcc/

exceeding ½ million bushels are quite common. Hence, it would take roughly 83 fully loaded trucks containing 100% high oleic soybean or multiple mistakes during harvest of high oleic soybean to achieve a 15% level of comingling.

Nutrition Facts Panel Labeling. Figure 3 and 4 demonstrate the amount of high oleic soybean oil that would be required to be comingled with commodity soybean oil in order to impact the Nutrition Facts Panel for major fatty acid categories. Based on this analysis, monounsaturated fat is the most sensitive fatty acid category and comingling up to 5% high oleic soybean oil could be tolerated before the Nutrition Facts Panel label for monounsaturated fatty acids were impacted on retail bottled vegetable oil. Up to 15% high oleic soybean could be comingled with commodity soybean oil before effects to polyunsaturated fatty acid labeling would be impacted.

The threshold for effects is lower than USP FCC labeling situation discussed above. In this case it would take a mistake during harvest of one acre of high oleic soybean comingled with 900 bushels of commodity soybean in a 900 bushel grain truck or 28 fully loaded trucks containing 100% high oleic soybean would need to be delivered to a ½ million bushel grain elevator containing commodity soybean to trigger an effect to the nutritional facts panel labeling.

The impact due to both of the comingling scenario discussed above would be minimal. As discussed previously, there would be no impacts to human health and the changes in fatty acids would benefit consumers from a nutritional perspective. Mandatory labeling is required for saturated fats, in this case comingling levels of up to 28% could be tolerated without impacting the level of saturated fats reported on the label. At 28%, the level of saturated fat would be lower, enhancing the nutritional profile of the product (Figure 4).

Oil Functionality; Severe Heat Processing. High oleic soybean oil has an improved stability profile compared to commodity soybean oil. An assessment of the impact of comingling of high oleic soybean oil with commodity soybean grain and oil was conducted. Information in Figure 5 demonstrates that comingling of high oleic soybean oil with commodity soybean oil increases the OSI stability index for commodity soybean oil. This outcome is expected since soybean oil oxidative stability is drastically influenced by the proportion of monounsaturates to polyunsaturates, and high oleic soybean oils are estimated to have improved oxidative stability compared to conventional soybean oil (Frankel, 2005).

Food Sensory Assessment. A sensory assessment of high oleic soybean oil was performed to evaluate consumer acceptability of high oleic soybean oil. Figure 6 demonstrates the sensory results for high oleic soybean oil in the most challenging environment high temperature food frying. In this experiment, commodity soybean oil and blends (5% to 15%) of commodity and high oleic soybean oil were used to prepare French fries. Food testers were asked to evaluate the quality of the fries using the Sensory Quality System described by King et al (2003). The outcome from the sensory evaluation showed that high oleic soybean oil comingled with commodity soybean oil blends were essentially the same as commodity

soybean oil over the six-day period during which the experiment was conducted. Thus, comingling of high oleic soybean oil with commodity soybean oil would have no impact to the sensory profile of commodity soybean oil should inadvertent comingling occur (Figure 6).

The comingling scenarios and impacts above are presented as the most conservative scenarios. Oilseed processors and users of vegetable oils are accustomed to the presence of numerous vegetable oils of differing fatty acid makeup that are available concurrently for blending and use in various food applications. The comingling levels described here where an impact could occur in functionality or labeling would happen only in instances where common control points were ignored. Such levels of comingling are highly unlikely to occur due to economic incentives to growers, legal contracts, stewardship SOP's (seed quality to end user), and demonstrated competency in managing inadvertent comingling with other vegetable oils that have a fatty acid profile similar to high oleic soybean oil. In addition, fatty acid analytical methods are widely available and used currently by oil processors and food manufacturers.

Industry Outreach

Monsanto has held conversations with the soybean and food industry key stakeholders mentioned above regarding the oil composition, stewardship plan and performance of MON 87705. Additionally, Monsanto has consulted with NOPA and industry members of NOPA's biotechnology committee as well as the ASA and USB regarding the fatty acid composition of high oleic soybean oil and potential changes to commodity soybean oil due to comingling. NOPA and members of their biotechnology committee were provided information related to the fatty acid composition of high oleic soybean oil derived from MON 87705 in the Vistive low-linolenic genetic background as well as oil properties and mixing effects information described previously. NOPA and biotechnology committee members agree that potential impacts related to the unintended mixing of commodity soybean oil and high oleic soybean oil could be remedied through blending, a common industry practice. Given the control points in the system and potential market impact of high oleic soybean oil, a risk mitigation plan (as described in Annex 2 of BIO's Product Launch Stewardship Policy) for high oleic soybean is not warranted. NOPA and the committee members continue to provide input on various aspects of the intended commercialization.

Summary

High oleic soybean offers an opportunity for soybean growers to recapture markets for vegetable oil previously occupied by soybean oil and create added value for U.S. soybean producers. Given the abundance of vegetable oils on the market and demonstrated ability of the system to adapt to consumer preferences incorporating new oils into existing food manufacturing processes, the market impact of high oleic soybean is expected to be minimal and easily managed. Monsanto has conducted and is implementing a product stewardship plan that is based

upon consideration of BIO's Launch Stewardship guidance and upon experience with previous successful product introductions completed by Monsanto. Monsanto continues to engage stakeholders and educate them on the benefits of high oleic soybean and proper stewardship practices.

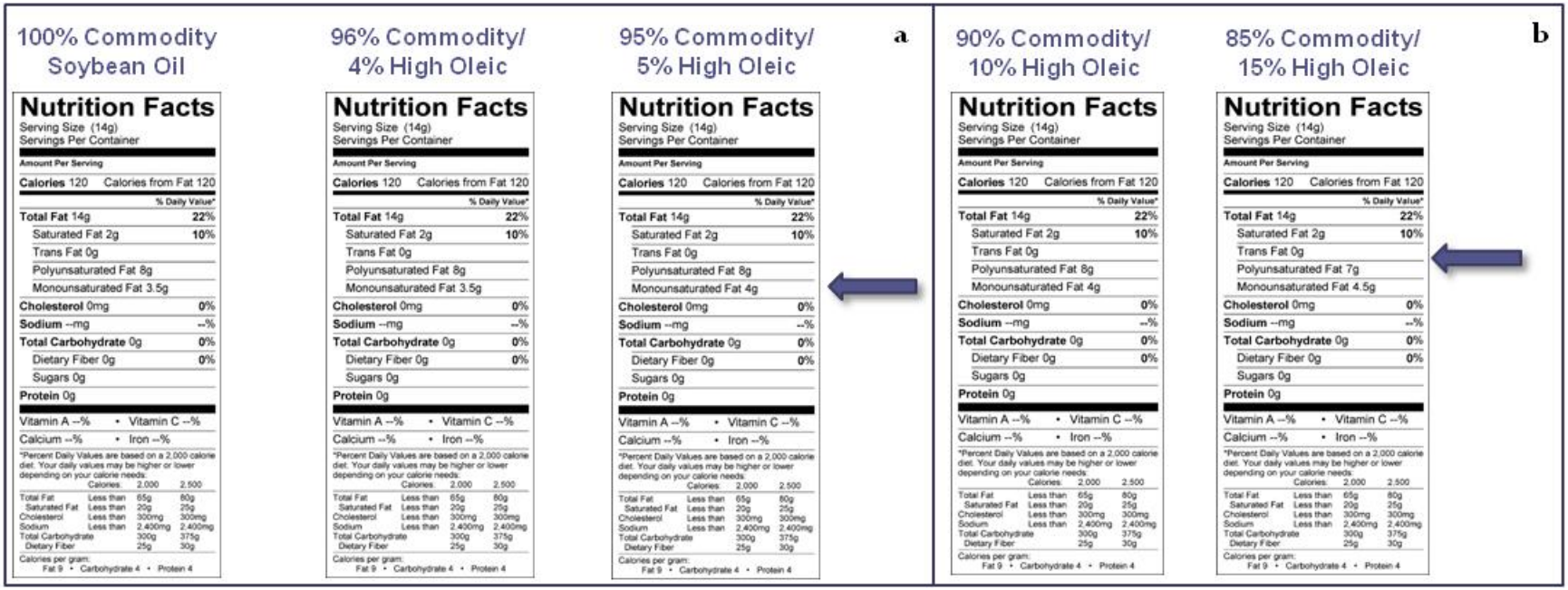
Table 4. Impact of comingling of high oleic soybean oil on Food Codex specifications of commodity soybean oil.

	USP FCC[1] Range SBO						Break Pt	
Major Fatty Acids	min	max	Typical Soy	Typical HO[2]	5% HO	10% HO	14% HO	15% HO
C16:0	7	12	10	2.49	10.071	9.672	9.3528	9.273
C18:0	2	5.5	4.64	3.34	4.575	4.51	4.458	4.445
C18:1	19	30	22.85	72.8	25.3475	27.845	29.843	30.3425
C18:2	48	65	52.7	16.6	50.895	49.09	47.646	47.285
C18:3 n3 ALA	5	10	6.97	3	6.7715	6.573	6.4142	6.3745

[1]FCC – Food Chemical Codex specification for named vegetable oils
[2]HO – Oil profile represents high oleic MON 87705 soybean bred into a low linolenic soybean background.

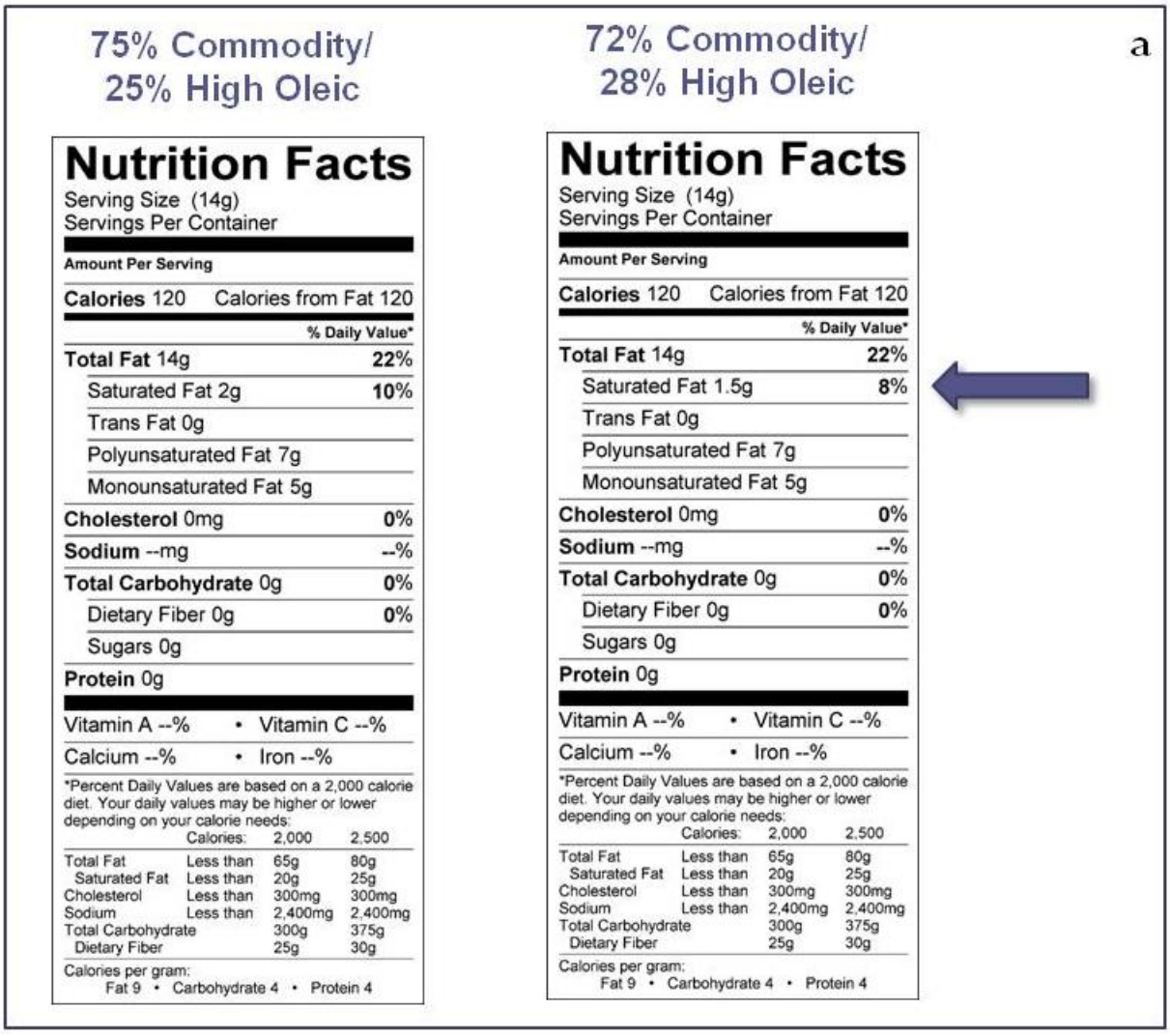
Legend. The impact of mixing high oleic soybean oil on the fatty acid profile of commodity soybean oil is depicted in the table above. The impact to the fatty acid profile was assessed at various percentages of high oleic soybean oil mixed with commodity soybean oil. The "break point" reflects the percentage of high oleic soybean oil at which the fatty acid levels in soybean oil would no longer be in the USP FCC specification range for soybean oil.

Figure 3. Impact of comingling high oleic soybean oil with bottled commodity soybeal oil on nutritional facts labeling to mono and polu unsaturated fatty acids.



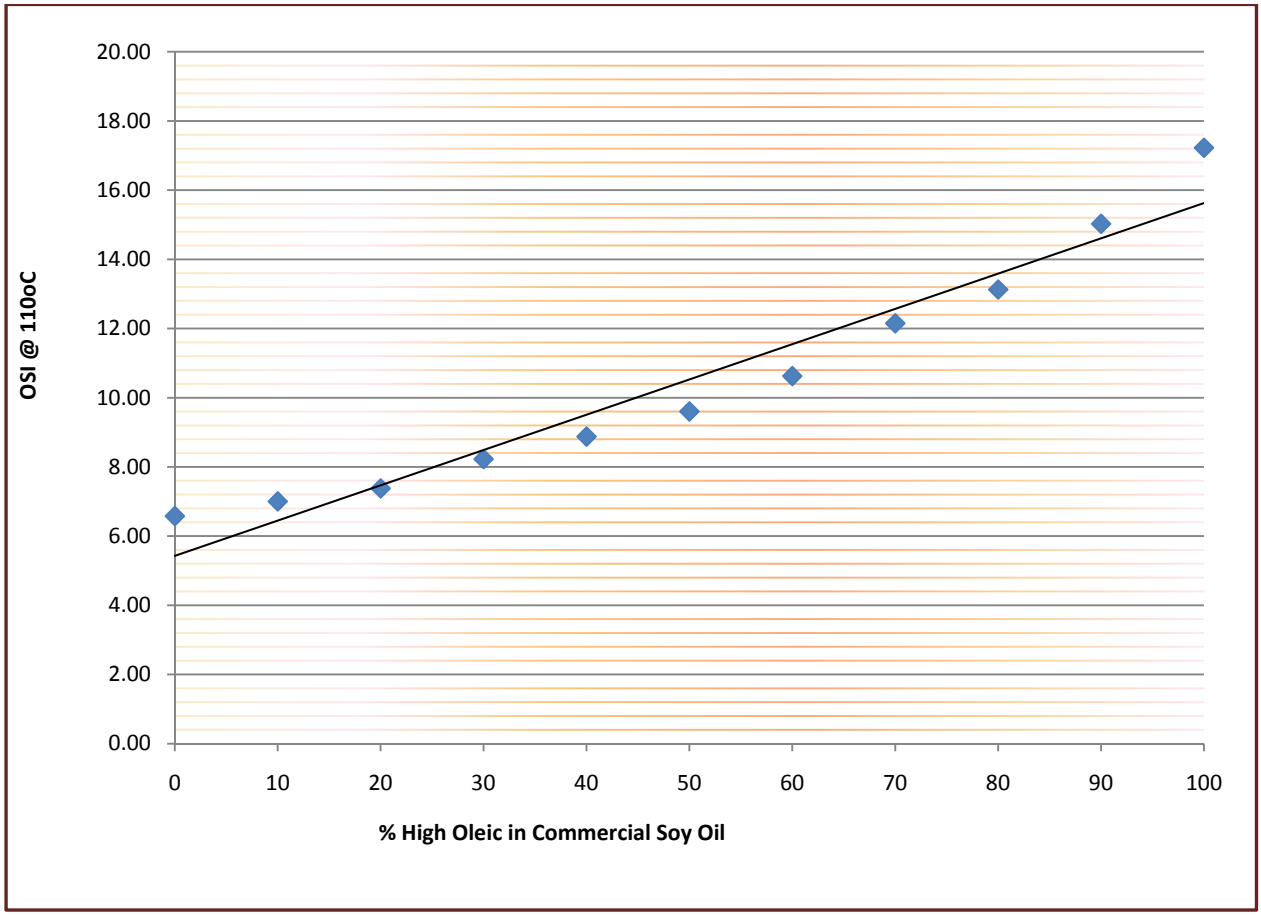
Legend. The impact of mixing high oleic soybean oil on nutritional facts labeling for bottled soybean oil is depicted in the figure above. The impact to nutritional facts panel labeling was assessed at various percentages of high oleic soybean oil mixed with commodity soybean oil. The blue arrows indicate the levels of high oleic soybean oil that would impact the nutritional facts labeling.

Figure 4. Impact of comingling high oleic soybean oil with bottled commodity soybeal oil on nutritional facts labeling to saturated fatty acids.



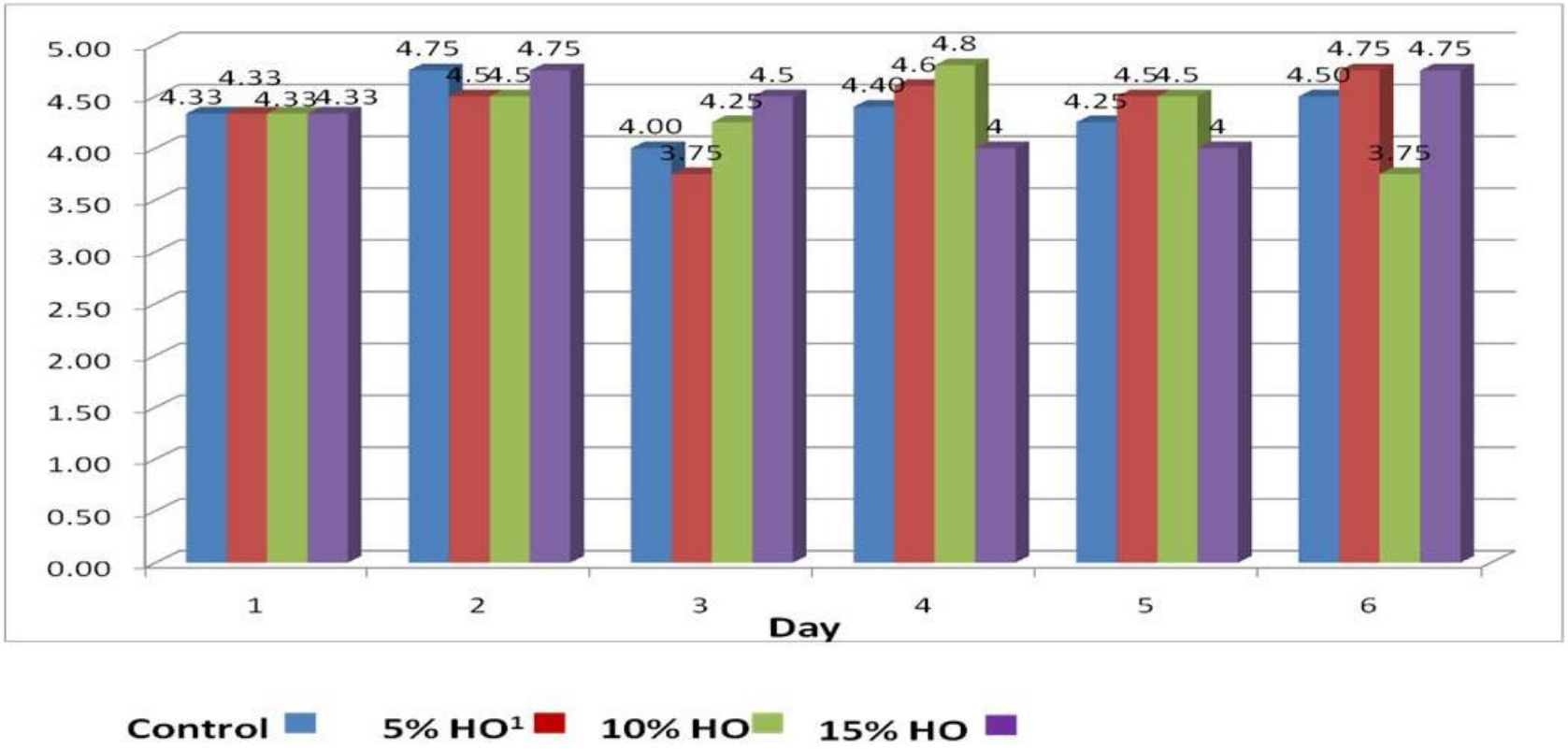
Legend. The impact of mixing high oleic soybean oil on nutritional facts labeling for bottled soybean oil is depicted in the figure above. The impact to nutritional facts panel labeling was assessed at various percentages of high oleic soybean oil mixed with commodity soybean oil. The blue arrows indicate the levels of high oleic soybean oil that would impact the nutritional facts labeling for mandatory fatty acid reporting (saturated fats).

Figure 5. Oxidative stability index of high oleic soybean blended with commodity soybean oil.



Legend. The impact of comingling of high oleic soybean oil was assessed on the oxidative stability index of the blended oil. The OSI stability index is an indicator of soybean oil stability. All oils and fats have a resistance to oxidation, which depends on the degree of saturation, antioxidant and prooxidant concentration, and prior abuse. Oxidation is slow until this resistance is overcome, at which point oxidation accelerates and becomes more rapid. The length of time prior to the acceleration of oxidation is referred to as the 'induction period,' and the point of maximum rate change is referred to as the Oxidative Stability Index or Oil Stability Index (OSI), and is reported in hours (Frankel, 2005).

Figure 6. Sensory assessment of high oleic soybean oil compared to other vegetable oils.



Legend. A sensory assessment of the quality of French fries prepared in blends of high oil/commodity soybean oil was conducted over a period of six days. A modification of the procedure described by King et al (2002) was used for the evaluation. The sensory qualities of the oil blends tested were essentially the same over the six-day period. Scale: 5 = no difference, 4 = very slight difference, 3 = slight difference, 2 = different, 1 = very different.

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ATTACHMENT I

THE MARKET POTENTIAL FOR A HIGH OLEIC SOYBEAN OIL

CONFIDENTIAL BUSINESS INFORMATION

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