Petition for the Determination of Nonregulated Status for Dicamba and Glufosinate-Tolerant Cotton MON 88701

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

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RELEASE OF INFORMATION

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

CERTIFICATION

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

[redacted]

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EXECUTIVE SUMMARY

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

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived cotton product, MON 88701, any progeny derived from crosses between MON 88701 and conventional cotton, and any progeny derived from crosses of MON 88701 with biotechnology-derived cotton that have previously been granted nonregulated status under 7 CFR Part 340.

Product Description

Monsanto Company has developed dicamba and glufosinate-tolerant cotton, MON 88701, which will allow in-crop applications of dicamba herbicide for the control of broadleaf weeds from preemergence to seven days preharvest and glufosinate herbicide for broad spectrum weed control from emergence through early bloom growth stage. MON 88701 provides a wider dicamba window of application beyond the current preplant cotton uses and glufosinate application rates and timings that are equivalent to current commercial glufosinate-tolerant cotton. The combination of these two unique herbicide modes-of-action provides an effective weed management system for cotton production. Dicamba provides effective control of over 95 annual and biennial weed species, and suppression of over 100 perennial broadleaf and woody plant species. Glufosinate, a broad-spectrum contact herbicide, provides nonselective control of approximately 120 broadleaf and grass weeds. Additionally, dicamba and glufosinate provide control of herbicide-resistant weeds, including glyphosate-resistant biotypes of Palmer amaranth (*Amaranthus palmeri*), marestail (*Conyza canadensis*), common ragweed (*Ambrosia artemisiifolia*), giant ragweed (*Ambrosia trifida*) and waterhemp (*Amaranthus tuberculatus*).

MON 88701 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide. DMO protein rapidly demethylates dicamba to the herbicidally inactive metabolite 3,6-dichlorosalicylic acid (DCSA). DCSA has been previously identified as a metabolite of dicamba in cotton, soybean, livestock, and soil. Monsanto will request a registration from U.S. EPA for the expanded use of dicamba on MON 88701, an increase in the dicamba residue tolerance for cottonseed, the establishment of a tolerance for cotton gin by-products, and the inclusion of DCSA in the residue definitions for both cottonseed and gin by-products. No other revisions to the dicamba pesticide residue tolerances are necessary including animal products such as meat, eggs, and milk.

Furthermore, the use of dicamba on MON 88701 does not present any new environmental exposure scenarios not previously evaluated and deemed acceptable by U.S. EPA.

MON 88701 also contains a bialaphos resistance (*bar*) gene from *Streptomyces hygroscopicus* that expresses the phosphinothricin N-acetyltransferase (PAT) protein to confer tolerance to glufosinate herbicide. PAT (*bar*)[1] protein acetylates the free amino group of glufosinate to produce non-herbicidal N-acetyl glufosinate, a well known metabolite in glufosinate-tolerant plants. The use pattern and rate of glufosinate on MON 88701 will follow the existing glufosinate-tolerant cotton uses outlined on the glufosinate herbicide label. The glufosinate residues in MON 88701 treated with commercial glufosinate rates are below the established pesticide residue tolerances for both cottonseed and gin by-products. Therefore, Monsanto will not seek any changes in the glufosinate label or the established tolerances for its use on MON 88701 cotton.

MON 88701 will be combined, through traditional breeding methods, with other deregulated herbicide-tolerant (*e.g.*, glyphosate-tolerant) events. The in-crop use of dicamba and glufosinate herbicides, in addition to glyphosate herbicide, provides improved weed management options in cotton to control a broad spectrum of grass and broadleaf weed species and effective control of weeds resistant to several herbicide families. Successful integration of MON 88701 into glyphosate-tolerant cotton systems will provide: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system for two additional herbicide modes-of-action for in-crop application in current cotton production systems as recommended by weed science experts to manage future weed resistance development; 3) an option to delay or prevent further resistance to glyphosate and other critically important cotton herbicides; in particular, herbicides in the acetolactate synthase inhibitor (ALS) and protoporphyrinogen oxidase inhibitor (PPO) class of chemistry; 4) crop safety to dicamba, glufosinate, and glyphosate; and 5) additional weed management tools to enhance weed management systems necessary to maintain yield and quality to meet the growing needs of the food, feed, and industrial markets.

Data and Information Presented Confirms the Lack of Plant Pest Potential and the Food and Feed Safety of MON 88701 Compared to Conventional Cotton

The data and information presented in this petition demonstrate MON 88701 is agronomically, phenotypically, and compositionally comparable to commercially cultivated cotton, with the exception of its tolerances to both dicamba and glufosinate. Moreover, the data presented demonstrate MON 88701 is unlikely to pose an increased plant pest risk, including weediness, or adverse environmental impact, compared to commercially cultivated cotton. The food, feed, and environmental safety of MON 88701 was confirmed based on multiple, well-established lines of evidence:

[1] PAT (*bar*) indicates the PAT protein encoded by the *bar* gene isolated from *S. hygroscopicus*. The *pat* gene from *S. viridochromogenes* also encodes a PAT protein that confers glufosinate tolerance.

- Cotton is a familiar crop that does not possess any of the attributes commonly associated with weeds, and has a history of safe usage and consumption.

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

- Extensive evaluation of the proteins expressed in MON 88701, dicamba mono-oxygenase (MON 88701 DMO) and phosphinothricin acetyltransferase [PAT (*bar*)], confirmed they are unlikely to be toxins or allergens. In addition, PAT proteins are in several other commercially-available crops that have been reviewed and previously deregulated by USDA, including those in cotton, corn, soy, canola, sugarbeet, and rice.

- A compositional assessment of cottonseed confirmed that MON 88701 is compositionally equivalent to commercially cultivated cotton.

- An extensive evaluation of phenotypic, agronomic, and plant mapping characteristics, as well as environmental interactions of MON 88701, demonstrated no increased plant pest potential compared to commercially cultivated cotton.

- An assessment of potential impact on non-target organisms (NTOs) indicated that, under anticipated agricultural conditions, MON 88701 is unlikely to have adverse effects on these organisms compared to commercially cultivated cotton.

- Evaluation of MON 88701 using current agronomic management practices for cotton concluded that deregulation of MON 88701 is not likely to impact cotton agronomic practices or land use, with the exception of the expanded window of dicamba application.

Cotton is a Familiar Crop Lacking Weedy Characteristics

Cotton, as a commodity crop, has a longstanding history of cultivation; its by-products, including processed fractions, also have a history of safe use and consumption. Cotton is grown in 17 states across the southern U.S. and in over 80 countries world-wide. In 2011, U.S. growers planted approximately 14.7 million acres of cotton.

The commercial cotton species in the U.S. (*Gossypium hirsutum* and *Gossypium barbadense* L. Merr.) do not exhibit weedy characteristics as defined by USDA, and neither invade established ecosystems, nor outcross to weedy relatives. Cotton is not listed as a weed in major weed references, nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Cotton does not possess any of the attributes commonly associated with weeds, such as long persistence of the seed in the soil, ability to disperse, invade, or become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. It is recognized that in some agricultural systems, cotton can volunteer in a subsequent rotational crop. However, volunteers are easily controlled through tillage or the use of appropriate

herbicides with diverse modes-of-action (*e.g.*, ALS inhibitor, chloroacetamide, EPSPS, PPO inhibitor, PSI disruption, PSII inhibitor, synthetic auxin, and tubulin inhibitor classes). Specificity studies using the aforementioned herbicides as potential substrates for MON 88701 DMO showed similar injury levels for MON 88701 compared to the conventional control, indicating that these herbicides do not serve as a substrate for MON 88701 DMO at commercial application rates. Additionally, the specificity of PAT (*bar*) has been established in the published scientific literature. Therefore, herbicides effective for control of volunteer conventional cotton can still be used to control MON 88701 volunteers.

In the continental U.S., wild populations of *Gossypium* species and some feral populations of cultivated variants of *G. hirsutum* exist, but these species able to cross with cultivated cotton are not known to exist in cotton growing areas. Importantly, MON 88701 would not be expected to confer a selective advantage to, or enhance the pest potential of, progeny resulting from such a cross if it were to occur, and could easily be controlled through current agronomic practices used to control conventional cotton. Thus, with environmental and biological limitations and varying chemical and agronomic practices available in the areas with wild and/or feral populations, there is limited probability for MON 88701 or any *Gossypium* species to outcross with wild or feral plants.

Conventional Cotton Coker 130 is an Appropriate Comparator to MON 88701

Cotton variety Coker 130 is the near isogenic line to MON 88701 and was used as the conventional cotton comparator to support the safety assessment of MON 88701. MON 88701 and the near isogenic conventional cotton control Coker 130 have similar genetic backgrounds with the exception of the *dmo* and *bar* expression cassettes; thus, the effect of the *dmo* and *bar* expression cassettes and the expressed MON 88701 DMO and PAT (*bar*) proteins could be evaluated.

Molecular Characterization Verified the Integrity and Stability of the Inserted DNA in MON 88701

MON 88701 was developed through *Agrobacterium*-mediated transformation of hypocotyls from cotton variety Coker 130 utilizing vector PV-GHHT6997. PV-GHHT6997 contains one T-DNA that is delineated by Left and Right Border regions. The T-DNA contains the *dmo* and *bar* expression cassettes. The *dmo* expression cassette is regulated by the *PCISV* promoter, the *TEV* 5′ leader sequence, and the *E6* 3′ untranslated region. The chloroplast transit peptide CTP2 directs transport of the MON 88701 DMO protein to the chloroplast and is derived from *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene. The *bar* expression cassette is regulated by the *e35S* promoter, the *Hsp70* leader, and the *nos* 3′ untranslated region. After transformation, self pollination and segregation were used to select those plants containing a single homozygous copy of the T-DNA, including both the *dmo* and *bar* expression cassettes, resulting in the selection of MON 88701.

Molecular characterization determined that MON 88701 contains one copy of the T-DNA at a single integration locus and all genetic elements are present. These data also demonstrated that MON 88701 does not contain detectable backbone sequences from the plasmid vector. The complete DNA sequence of the insert and adjacent genomic DNA sequences in MON 88701 confirmed the integrity of the inserted *dmo* and *bar* expression cassettes and identified the 5′ and 3′ insert to flank DNA junctions. Molecular characterization analysis also demonstrated that the insert in MON 88701 has been maintained over five consecutive generations of breeding, thereby confirming the stability of the insert. Furthermore, results from segregation analyses show inheritance and stability of the insert were as expected across multiple generations, which corroborates the molecular insert stability analysis determination that the MON 88701 T DNA resides at a single chromosomal locus within the cotton genome.

Data Confirms MON 88701 DMO and PAT (*bar*) Protein Safety

A multistep approach was used to characterize and assess the safety of the MON 88701 DMO and PAT (*bar*) proteins expressed in MON 88701 resulting from the genetic modification. The expression levels of the MON 88701 DMO and PAT (*bar*) proteins in selected tissues of MON 88701 were determined. An assessment of the allergenic potential of the MON 88701 DMO and PAT (*bar*) proteins supports the conclusion that neither protein poses a significant allergenic risk to humans or animals. In addition, the donor organisms for the MON 88701 DMO and PAT (*bar*) protein coding sequences, *Stenotrophomonas maltophilia* and *Streptomyces hygroscopicus*, respectively, are ubiquitous in the environment and are not commonly known for human or animal pathogenicity or allergenicity. Bioinformatics analysis determined that the MON 88701 DMO and PAT (*bar*) proteins lack structural similarity to known allergens, gliadins, glutenins, or protein toxins. The MON 88701 DMO and PAT (*bar*) proteins are rapidly digested in simulated gastrointestinal fluids and neither protein demonstrates acute oral toxicity in mice at the levels tested. Hence, the consumption of the MON 88701 DMO and PAT (*bar*) proteins from MON 88701 or its progeny poses no meaningful risk to the environment or human and animal health.

MON 88701 is Compositionally Equivalent to Conventional Commercial Cotton

Detailed compositional analyses were conducted in accordance with OECD guidelines to assess whether levels of key nutrients and anti-nutrients in MON 88701 cottonseed were comparable to levels in the conventional control, Coker 130, and several commercial reference cotton varieties. These compositional comparisons were made by analyzing cottonseed harvested from eight U.S. field sites in which MON 88701 was treated with dicamba and glufosinate, with the conventional control, and a range of commercial reference varieties that were grown concurrently in the same field trial. Compositional comparisons of MON 88701 not treated with dicamba or glufosinate herbicides were also conducted to further support the assessment of MON 88701 traits. The commercial reference varieties used to establish a range of natural variability for key nutrients and anti-nutrients have a history of safe consumption. Nutrients assessed in this analysis included proximates (ash, carbohydrates, and calories by calculation, moisture, protein, and fat), fibers (ADF, crude fiber, NDF, and TDF), amino acids (18 components), fatty

acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) and vitamin E. The anti-nutrients assessed in this analysis included gossypol and the cyclopropenoid fatty acids dihydrosterculic, malvalic, and sterculic.

Combined-site analyses were conducted to determine if there were any statistically-significant differences (5% level of significance) between MON 88701 and the conventional control cottonseed samples. Significant differences noted from the combined-site statistical comparison were assessed using considerations relevant to the safety and nutritional quality of MON 88701 when compared to the conventional control. Considerations used to assess the relevance of each combined-site statistically significant difference included: 1) the relative magnitude of the difference in the mean values of nutrient and anti-nutrient components between MON 88701 and the conventional control; 2) whether the MON 88701 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of the commercial reference varieties grown concurrently in the same trial; 3) evaluation of the reproducibility of the statistical ($p < 0.05$) combined-site component differences at individual sites; and 4) an assessment of the differences within the context of natural variability of commercial cotton composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database.

Based on these criteria, the observed differences were not meaningful to food and feed safety or nutritional value, and led to the conclusion that MON 88701 is compositionally equivalent to commercially cultivated cotton that has a history of safe consumption. These results support the overall food and feed safety of MON 88701.

MON 88701 Does Not Change Cotton Plant Pest Potential or Environmental Interactions

Plant pest potential of a biotechnology-derived crop is assessed from the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant hybrid or variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait(s), the receiving environment, and the interactions among these factors. This provides a basis for comparative risk assessment between a biotechnology-derived plant and the conventional control. Thus, the phenotypic, agronomic, plant mapping, and environmental interaction assessment of MON 88701 included the parental conventional control as a comparator. This evaluation used a weight-of-evidence approach and considered statistical differences between MON 88701 and the conventional control with respect to reproducibility, magnitude, and directionality. The observations were taken on plants not treated with dicamba or glufosinate, in order to evaluate the impact of the introduced traits in MON 88701. To further support the trait assessment, similar supplemental observations were also conducted on the agronomic system that includes MON 88701 treated with dicamba and glufosinate herbicides. Comparison to a range of commercial reference varieties established the range of natural variability for cotton, and provided a context from which

to further evaluate any statistical differences. Characteristics assessed included: seed dormancy and germination, pollen morphology, plant phenotypic observations, plant mapping, and environmental interaction evaluations conducted in the field. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 88701 is comparable to conventional cotton. Thus, MON 88701 is unlikely to have increased weediness or plant pest potential compared to commercially cultivated cotton.

Seed dormancy and germination characterization demonstrated that MON 88701 cottonseed had germination characteristics similar to cottonseed of the conventional control. In particular, the lack of hard seed, a well-accepted characteristic of weediness affecting seed germination, supports a conclusion of no increased weediness of MON 88701 when compared to the conventional control. Additionally, there were no statistically significant (5% level of significance) differences observed between MON 88701 and the conventional control for pollen viability and diameter, and no visual differences in general pollen morphology were observed. Collectively, these results support the conclusion that MON 88701 is not likely to exhibit increased plant pest potential compared to commercially cultivated cotton.

The field evaluation of phenotypic, agronomic, plant mapping, and environmental interaction characteristics of MON 88701 also support the conclusion that MON 88701 is not likely to have an increased plant pest potential compared to commercially cultivated cotton. The evaluations were conducted at 26 replicated field sites across the U.S. cotton producing region. These assessments included plant growth and development characteristics, including cotton plant mapping evaluations at harvest, as well as observations for plant responses to abiotic stressors and plant-disease and plant-arthropod interactions. The observed phenotypic characteristics were similar between MON 88701 and the conventional control.

In a combined-site analysis of plant growth and development characteristics, data showed no statistically significant differences (5% level of significance) between MON 88701 and the conventional control for stand count at 14 and 30 days after planting (DAP), final stand count, number of nodes above white flower at one of three observations, seed cotton yield, immature seed per boll, weight per boll, micronaire, fiber elongation, fiber uniformity, and fiber length. The mean values for MON 88701 were statistically different from the conventional control for eight parameters in the combined-site analysis. MON 88701 had shorter plants at 30 DAP and harvest, an increased number of nodes above white flower at two observations, a lower seed index, increased seed per boll, increased mature seeds per boll, and increased fiber strength. However, the mean values of MON 88701 were within the range of values observed for the commercial reference varieties for each of the characteristics listed above. Therefore, none of these differences were considered biologically meaningful in terms of increased plant pest potential of MON 88701 compared to commercially cultivated cotton.

Plant mapping is a process commonly used by cotton agronomists and breeders to quantify growth and development parameters of a cotton plant, including boll retention. Plant mapping parameters, which include delineation of boll position and spatial retention

of bolls, are used to measure crop productivity and are influenced by abiotic and biotic stressors. In the combined-site analysis of plant mapping parameters, no statistically significant differences were detected between MON 88701 and the conventional control for number of mainstem nodes, number of nodes to first fruiting branch, total number of bolls per plant, number of vegetative bolls per plant, percent retention of first-position bolls, and percent first-position bolls. One statistically significant difference was detected between MON 88701 and the conventional control in the combined-site analysis. The mean value for first-position bolls per plant was higher for MON 88701 than the conventional control. However, the mean value of the number of first-position MON 88701 bolls was within the range of the commercial reference varieties. Thus, MON 88701 is similar to commercially cultivated cotton varieties and unlikely to have increased plant pest potential, increased weediness, or an adverse environmental impact compared to commercially cultivated cotton.

In an individual site assessment of abiotic stress response and disease damage, no differences were observed between MON 88701 and the conventional control for any of the 296 comparisons for the assessed abiotic stressors or for any of the 299 comparisons for the assessed diseases among all observations at the 26 sites. In an assessment of arthropod-related damage, no differences were detected between MON 88701 and the conventional control for any of the 288 comparisons for the assessed arthropods. The lack of significant biological differences in plant responses to abiotic stress, disease damage, and arthropod-related damage for MON 88701 support the conclusion that the introduction of the dicamba and glufosinate tolerance traits are unlikely to result in increased plant pest potential or an altered environmental impact from MON 88701 compared to commercially cultivated cotton.

In an assessment of pest- and beneficial-arthropod abundance, no statistically significant differences (5% level of significance) were detected between MON 88701 and the conventional control for 173 out of 178 comparisons (including 89 arthropod-pest and 89 beneficial-arthropod comparisons) among the multiple collections conducted during the season at five geographically diverse sites. For the five detected differences in arthropod abundance, two were arthropod pests (stink bugs and tarnished plant bugs) and three were beneficial arthropods (*Nabis* spp. and *Orius* spp.). The differences detected in pest- and beneficial-arthropod abundance were small in magnitude and were not consistent with other collections at the individual sites or across the sites. Consequently, it is concluded that the differences in pest- and beneficial-arthropod abundance are not indicative of a consistent plant response associated with MON 88701 and are not biologically meaningful in terms of increased plant pest potential or an altered environmental impact from MON 88701 compared to commercially cultivated cotton.

Field evaluations of phenotypic, agronomic, and plant mapping characteristics of MON 88701 treated with dicamba and glufosinate herbicides were also conducted to further support the assessment of MON 88701 traits. Data were collected from field trials conducted at eleven sites within the U.S. cotton-producing region. These assessments included plant growth and development characteristics, as well as plant mapping evaluations at harvest. The phenotypic, agronomic, and plant mapping assessments demonstrated that herbicide-treated MON 88701 is not different than the

conventional control, which further supports that MON 88701, whether treated or not with dicamba and glufosinate, is unlikely to have an altered plant pest potential compared to commercially cultivated cotton.

In summary, the phenotypic, agronomic, plant mapping and environmental interaction data were evaluated to characterize MON 88701, and to assess whether the introduction of the traits in MON 88701 alters the plant pest potential compared to conventional cotton. The evaluation, using a weight-of-evidence approach, considered the reproducibility, magnitude, and direction of detected differences between MON 88701 and the conventional control, and comparison to the range of the commercial reference varieties. Results from the phenotypic, agronomic, plant mapping, and environmental interactions assessment indicated that MON 88701 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant environmental impact compared to commercially cultivated cotton.

MON 88701 Will Not Adversely Affect NTOs

Evaluation of the impacts of a biotechnology-derived crop on non-target organisms (NTOs) is a component of the plant pest risk assessment. Since MON 88701 does not possess pesticidal activity, all organisms that interact with MON 88701 are considered to be NTOs. The environmental assessment demonstrated that the presence of the dicamba and glufosinate-tolerance traits in MON 88701 did not alter plant-arthropod interactions, including beneficial arthropods, or alter disease susceptibility compared to the conventional control. In addition, plant mapping data, which is utilized to determine crop productivity in relation to abiotic and biotic stresses affecting yield, demonstrated that both MON 88701 plots treated and not treated with dicamba and glufosinate herbicides each had only a single significant difference from the conventional control, an increased number of first-position bolls that was within the range of the commercial reference varieties. From these data it can be concluded that both MON 88701 plants treated and not treated with dicamba and glufosinate responded to stressors in a similar manner.

The biochemical information and experimental data for evaluation of MON 88701 included molecular characterization, MON 88701 DMO and PAT (*bar*) safety assessments, the history of environmental exposure to mono-oxygenases (the class of enzymes to which MON 88701 DMO belongs) and the PAT proteins in several commercial glufosinate-tolerant events, information from the environmental interaction assessment, demonstration of compositional equivalence to conventional cotton, and demonstration of agronomic and phenotypic equivalence to conventional cotton. Overall, these data support the conclusion that MON 88701 has no reasonable mechanism for harm to NTOs and does not pose any additional risk to NTOs compared to commercially cultivated cotton.

The potential for outcrossing and gene introgression from MON 88701 to sexually compatible species in the U.S. is unlikely, since the only known wild *Gossypium* species related to cultivated cotton do not grow in areas where cotton is cultivated, cotton pollen movement by wind is limited due to it is large and sticky nature, and several studies have

demonstrated that cross-pollination, even in the presence of high pollinator activity is limited by distance. Furthermore, should cross-pollination occur, MON 88701 and its progeny are not expected to exhibit a significant environmental impact because, as described above, evaluations have shown that the presence of the dicamba and glufosinate-tolerance traits are not likely to enhance weediness or plant-pest potential. Therefore, the environmental consequence of pollen transfer from MON 88701 to other *Gossypium* species is considered negligible.

Deregulation of MON 88701 is Not Likely to Impact Cotton Agronomic Practices or Land Use

Cotton fields are typically highly managed agricultural areas that are dedicated to crop production for many years. Cultivation of MON 88701 would not be expected to differ from typical cotton cultivation, with the sole exception of an expanded window of dicamba application, due to the presence of the dicamba-tolerance trait in MON 88701. As glufosinate is already utilized within the U.S. cotton-growing areas, no change in agronomic practices or land use would occur with the cultivation of MON 88701 and the presence of the glufosinate-tolerance trait. MON 88701 likely would be used in common rotations on land currently used for agricultural purposes. As demonstrated, MON 88701 is similar to commercially cultivated cotton in its agronomic, phenotypic, ecological, and compositional characteristics, and has comparable levels of resistance to insects, diseases, and abiotic stresses as compared to commercial cotton. Therefore, the introduction of MON 88701 into the existing cotton system is not expected to have a significant impact on current cultivation and pest management practices for cotton. The adoption of MON 88701 into glyphosate-tolerant cotton systems will provide growers with two additional herbicide modes-of-action and the means to control broadleaf weeds, including hard-to-control and herbicide-resistant broadleaf weeds, and will help preserve conservation tillage practices by providing growers with an additional weed management tool. Based on these considerations, MON 88701 is not likely to impact agronomic practices or land use, with the exception of the expanded application window of dicamba.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 88701 is not likely to be a plant pest. Therefore, Monsanto Company requests a determination from USDA-APHIS that MON 88701 and any progeny derived from crosses between MON 88701 and conventional *Gossypium* cotton species or deregulated biotechnology-derived cotton be granted nonregulated status under 7 CFR Part 340.

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ABBREVIATIONS AND DEFINITIONS[2]

Symbol or Abbrev.	Definition
~	Approximately
α-Cyano	α-Cyano-4-hydroxycinnamic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
2,4-DB	2,4-DB = 4-(2,4-dichlorophenoxy)butyric acid
AA	Amino Acid
AAbA	α-aminobutyric acid
ADF	Acid Detergent Fiber
a.e.	acid equivalent
a.i.	active ingredient
ALS	acetolactate synthase inhibitor
APHIS	Animal and Plant Health Inspection Service of the United States Department of Agriculture
bar	Bialaphos Resistance Gene from *Streptomyces hygroscopicus*
BIO	Biotechnology Industry Organization
BLOCKS	A database of amino acid motifs found in protein families
BLOSUM	Blocks Substitution Matrix, used to score similarities between pairs of distantly related protein or nucleotide sequences
BSA	Bovine Serum Albumin
CFR	Code of Federal Regulations
CHT	Ceramic hydroxyapatite
CoA	Coenzyme A
COA	Certificate of Analysis
CTAB	Hexadecyltrimethylammonium bromide
DAP	Days After Planting
Da	Dalton
dCTP	Deoxycytidine triphosphate
DEAE-	Diethylaminoethyl-
DHB	2,5-dihydroxybenzoic acid
DCSA	3,6-dichlorosalicylic acid
DDI	Daily Dietary Intake
DGA	Diglycolamine
dicamba	3,6-dichloro-2-methoxybenzoic acid
dmo	Mono-oxygenase gene from *Stenotrophomonas maltophilia*
DMO	Dicamba mono-oxygenase
DNA	Deoxyribonucleic acid
DSMA	Disodium methanearsonate
DTNB	5,5'-dithio-bis (2-nitrobenzoic acid)
DTT	Dithiothreitol
dw	Dry weight
DWCF	Dry weight conversion factor

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

ECL	Enhanced Chemiluminescence
E. coli	*Escherichia coli*
E.coli-produced MON 88701 DMO	DMO protein produced from *E. coli* with the same sequence as MON 88701 DMO
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Environmental Protection Agency
E-Score	Expectation score
ETS	Excellence Through Stewardship[SM]
FA	Fatty Acid
FARRP	Food Allergy Research and Resource Program
FASTA	Algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences
FDA	Food and Drug Administration (U.S.)
FFDCA	Federal Food, Drug and Cosmetic Act (U.S.)
FT	Flow through
fw	Fresh weight
glufosinate	butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)
GLP	Good Laboratory Practice
ha	hectare
HPLC	High Performance Liquid Chromatography
HRP	Horseradish Peroxidase
HU	Hemagglutinating Unit
ILSI	International Life Sciences Institute
ISO	International Organization for Standardization
kb	Kilobase
kDa	Kilodalton
kg	Kilogram
LB	Laemmli buffer
LOD	Limit of Detection
LOQ	Limit of Quantitation
MALDI-TOF-MS	Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry
µg	Microgram
µl	Microliter
mg	Milligram
mic	micronaire
MOE	Margin of Exposure
MON 87708	Dicamba-tolerant soybean developed by Monsanto Company
MON 88701 DMO	DMO protein produced in MON 88701
MRL	Maximum Residue Levels
MSMA	Monosodium methanearsonate
MW	Molecular Weight
MWCO	Molecular Weight Cutoff
N-acetyl glufosinate	2-acetamido-4-methylphosphinico-butanoic acid
NADH	Nicotinamide adenine dinucleotide

NCBI	National Center for Biotechnology Information at the National Institutes of Health, Bethesda, MD, USA
NDF	Neutral Detergent Fiber
NFDM	Non-fat Dried Milk
NOAEL	No Observable Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame
OSL	Overseason Leaf
p	Probability from PRESS
PAT	Phosphinothricin N-acetyltransferase
PAT (*bar*)	PAT protein produced by the *bar* gene
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline containing Tween-20
PCR	Polymerase Chain Reaction
PI	Prediction Interval
PPA	Plant Protection Act (7 U.S.C. § 7701-7772)
ppm	parts per million
PPO	protoporphyrinogen oxidase inhibitor
PPT	Phosphinothricin
PRESS	Predicted Residual Sum of Squares
PRT_2011	GenBank protein database, 181.0 (Released December 18, 2010)
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride
PVP	Polyvinyl pyrrolidone
RBD	Refined, Bleached, and Deodorized
RED	Reregistration Eligibility Decision
RT	Room temperature
SCST	Society of Commercial Seed Technologists
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SE	Standard Error
SGF	Simulated Gastric Fluid
S. hygroscopicus	*Streptomyces hygroscopicus*
SIF	Simulated Intestinal Fluid
Sinapinic Acid	3,5-dimethoxy-4-hydroxycinnamic acid
S. maltophilia	*Stenotrophomonas maltophilia*
SOP	Standard Operating Procedure
TBA	Tris-borate buffer with L-ascorbic acid
TBS	Tris Buffered Saline
TCEP	Tris(2-carboxyethyl)phosphine
T-DNA	Transfer DNA
TDF	Total Dietary Fiber
tex	Grams of 1000 meters of fiber
TFA	Trifluoroacetic Acid
TFE	2,2,2,-trifluoroethanol
TIU	Trypsin Inhibitor Unit

Tm	Melting temperature
TNB	5-thio-nitrobenzoate
TOX_2011	Toxin protein sequence database (Release date February 18, 2011)
V	volts
v/v	volume to volume ratio
w/v	weight to volume ratio

I. RATIONALE FOR THE DEVELOPMENT OF MON 88701

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

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

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived cotton product, MON 88701, any progeny derived from crosses between MON 88701 and conventional cotton, and any progeny derived from crosses of MON 88701 with biotechnology-derived cotton that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of Dicamba and Glufosinate-Tolerant Cotton – MON 88701

Biotechnology derived cotton and the introduction of glyphosate-tolerant cotton systems permit in-crop application of agricultural herbicides containing the active ingredient glyphosate for effective weed control. The value of glyphosate-tolerant cotton systems has been demonstrated by the significant growth in the number of glyphosate-tolerant acres planted since introduction of this technology in 1997. Today, more than 75% of all cotton acres grown in the U.S. are glyphosate-tolerant (USDA-NASS, 2010). The glyphosate-tolerant systems deliver effective broad spectrum weed control, provides flexibility of application timing, increased adoption of reduced tillage practices, and has resulted in increased grower income (Carpenter and Gianessi, 2001; Hurley et al., 2009). Additionally, the glyphosate-tolerant systems provide incremental environmental benefits, including reduced overall herbicide usage (Brookes and Barfoot, 2012; Carpenter and Gianessi, 2001). Furthermore, glyphosate, as concluded by the U.S. EPA (1993), has a favorable safety profile. Continued use of glyphosate-tolerant cotton systems will maintain effective and familiar weed control management practices that are fully compatible with all current tillage and land management practices, including conservation tillage practices. Growth of conservation tillage in the U.S. was greatly accelerated with the introduction of glyphosate-tolerant crops in large part because of the broad spectrum postemergence control offered by glyphosate (Price et al., 2011). The benefits associated with conservation tillage, include reduced soil erosion, reduced fuel and labor costs, and conservation of soil moisture (CTIC, 2011).

As with all herbicides used in agriculture, there is potential for weeds to develop resistance to frequent and continual use of the same herbicide over an extended time

period (Powles, 2008). Plant populations can develop resistance to a herbicide due to the selection of individuals that carry altered genetic codes producing alleles that can render those individuals tolerant to the lethal effects of a herbicide. Weed populations with confirmed herbicide-resistance are listed on the International Survey of Resistant Weeds website (www.weedscience.org). Without effective weed management practices in agricultural systems, herbicide resistance in weeds can become a limiting factor in crop production. As with many agricultural use herbicides, glyphosate has documented cases of weed resistance. While there have been thirteen confirmed glyphosate-resistant weeds in the U.S. (Heap, 2012a), glyphosate still effectively controls more than 160 weed species (Roundup WeatherMax® herbicide label, EPA Reg. No.524-537) and remains an extremely valuable tool for U.S. cotton crop production. Studies have shown that resistance can be postponed, contained, and managed through good management practices. One of the management practices most often recommended by University/Cooperative Extension Service and industry is the use of multiple herbicide modes-of-action. Simultaneously using multiple herbicides with different modes-of-action significantly reduces the probability of weeds developing resistance to any or all of the applied herbicides (Beckie and Reboud, 2009; Powles et al., 1996). Other weed management recommendations include the use of multiple herbicide modes-of-action in sequence and the inclusion of mechanical or cultural weed management practices, in addition to the use of a herbicide.

Monsanto Company has developed dicamba and glufosinate-tolerant cotton, MON 88701, which will allow in-crop applications of dicamba (3,6-dichloro-2-methoxybenzoic acid) herbicide for the control of broadleaf weeds from preemergence to seven days preharvest and glufosinate herbicide for broad spectrum weed control from emergence through early bloom growth stage. MON 88701 provides dicamba tolerance that allows for the in-crop application of dicamba beyond the current preplant uses in cotton and also provides glufosinate tolerance equivalent to current commercial glufosinate-tolerant cotton events. The combination of the two herbicides' distinct modes-of-action provides an effective weed management system. Dicamba provides effective control of over 95 annual and biennial weed species, and suppression of over 100 perennial broadleaf and woody plant species (BASF, 2008) (EPA Reg. No. 7969-137) and glufosinate is a broad-spectrum contact herbicide that provides nonselective control of about 120 broadleaf and grass weeds (Bayer CropScience, 2011) (EPA Reg. No. 264-829). Additionally, dicamba and glufosinate each provide control of many herbicide-resistant weeds, including glyphosate-resistant biotypes of Palmer amaranth (*Amaranthus palmeri*), marestail (*Conyza Canadensis*), common ragweed (*Ambrosia artemisiifolia*), giant ragweed (*Ambrosia trifida*) and waterhemp [*Amaranthus tuberculatus*). Weeds that are hard-to-control using glyphosate (See Roundup WeatherMax® label (U.S. EPA Reg. No. 524-537) for a listing], generally require a higher rate and/or application at a smaller growth stage in order to consistently achieve commercially acceptable control. To date, only four species with known dicamba-resistant biotypes (*i.e.*, common hempnettle, *Galeopsis tetrahit*; kochia, *Kochia scoparia*; prickly lettuce, *Lactuca serriola*; and wild mustard, *Sinapis arvensis*) and one species

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with a known glufosinate-resistant biotype (*i.e.*, Italian ryegress, *Lolium multiflorum*) have been identified in North America (Heap, 2012b; 2012c). Known resistant weed populations to dicamba and glufosinate are primarily found in the western U.S. and, thus, are not present in the major cotton geographies. See Appendix I for additional details.

MON 88701 will be combined, through traditional breeding methods, with other approved herbicide-tolerant (*e.g.*, glyphosate-tolerant) events. The opportunity for in-crop use of dicamba and glufosinate herbicides, in addition to glyphosate herbicide, provides new weed management options in cotton to control a broad spectrum of grass and broadleaf weed species and effective control of weeds resistant to several herbicide families. Successful integration of MON 88701 into glyphosate-tolerant cotton systems will provide: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system for two additional in-crop herbicide modes-of-action in current cotton production practices as recommended by weed science experts to manage future weed resistance development; 3) an option to delay or prevent further resistance to glyphosate and other critically important cotton herbicides, in particular herbicides in the ALS and PPO class of chemistry; 4) crop safety to dicamba, glufosinate and glyphosate; and 5) additional weed management tools to enhance weed management systems necessary to maintain yield and quality to meet the growing needs of fiber, food, and feed.

MON 88701 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide and a bialaphos resistance (*bar*) gene from *Streptomyces hygroscopicus* that expresses the phosphinothricin N-acetyltransferase (PAT) protein to confer tolerance to glufosinate herbicide. DMO protein rapidly demethylates dicamba to the herbicidally inactive metabolite 3,6-dichlorosalicylic acid (DCSA), a well known metabolite of dicamba in conventional cotton, soybean, livestock, and soil (FAO-WHO, 2011a; 2011b; U.S. EPA, 2009). Monsanto will request a registration from U.S. EPA for the expanded use of dicamba on MON 88701 cotton, an increase in the dicamba residue tolerance for cottonseed, the establishment of a tolerance for cotton gin by-products, and the inclusion of DCSA in the residue definitions for cottonseed and gin by-products. No other revisions to the dicamba pesticide residue tolerances are necessary, including those for animal products such as meat, eggs, and milk. Furthermore, the use of dicamba on MON 88701 does not present any new environmental exposure scenarios not previously evaluated and deemed acceptable by EPA.

PAT (*bar*) protein acetylates the free amino group of glufosinate to produce non-herbicidal N-acetyl glufosinate, a well known metabolite in glufosinate-tolerant plants (OECD, 2002a). The use pattern and rate of glufosinate on MON 88701 will follow the existing glufosinate-tolerant cotton uses outlined on the glufosinate herbicide labels and the glufosinate residues in MON 88701 treated with commercial glufosinate rates are below the established pesticide residue tolerances established by U.S. EPA for both cottonseed and gin by-products (40 CFR § 180.473). Therefore, Monsanto will not pursue any changes in the glufosinate labels or the established tolerances for its use on MON 88701 cotton.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology (CFR) (USDA-APHIS, 1986), the responsibility for regulatory oversight of biotechnology-derived crops falls primarily on three U.S. agencies: U.S. Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and in the case of herbicide-tolerant products, the Environmental Protection Agency (EPA). A request for deregulation of MON 88701 made to USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 88701 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions. Additionally, EPA must complete its review and assessments prior to approving the use and allowable residues of dicamba on MON 88701.

I.C.1. Submission to FDA

MON 88701 falls within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA, 1992). In compliance with this policy, Monsanto has initiated a consultation with the FDA (BNF No. 135) on the food and feed safety and compositional assessment of MON 88701. Monsanto submitted a safety and nutritional assessment summary document to the FDA in April 2012.

I.C.2. Submission to EPA

The safety of dicamba use on many crops, including cotton, was reviewed by the Environmental Protection Agency (EPA) as part of the food, feed, and environmental safety reassessment in 2006 (U.S. EPA, 2009). Dicamba can currently be applied to cotton in the U.S. as a pre-plant application, at least 21 days prior to planting. The tolerance of MON 88701 to dicamba facilitates a wider window of application on cotton, allowing pre-emergence application of the herbicide up to the day of crop emergence and post-emergence in-crop applications through seven days pre-harvest. Monsanto will request a registration from U.S. EPA for the expanded use of dicamba on MON 88701, an increase in the dicamba residue tolerance from 0.2 ppm to 3 ppm for cottonseed, the establishment of a tolerance of 70 ppm for cotton gin by-products, and the inclusion of DCSA in the residue definitions for cottonseed and gin by-products. No other revisions to dicamba pesticide residue tolerances are needed including animal products such as meat, eggs, or milk.

The existing 0.2 ppm pesticide residue tolerance for cottonseed supporting the current registered uses of dicamba on cotton (40 CFR § 180.227) is for the combined residues of parent dicamba and its metabolite 5-hydroxy dicamba. Cotton gin by-products, a ruminant feed supplement, have no established dicamba tolerance. Studies have shown that the proposed use of dicamba on MON 88701 cotton results in total residue concentrations of parent dicamba and its metabolites, including DCSA and 5-hydroxy dicamba, are less than 3 ppm for cottonseed and less than 70 ppm for gin by-products.

The safety of glufosinate use on many crops, including cotton, was reviewed by the Environmental Protection Agency (EPA) as part of the food, feed, and environmental safety reassessment in 2000 (U.S. EPA, 2003). In addition, glufosinate has been used over-the-top of glufosinate-tolerant crops since 1995 with no significant adverse effects reported. Glufosinate is currently labeled for in-crop application on glufosinate-tolerant cotton from emergence through early bloom growth stage (Bayer CropScience, 2011). The use pattern and rate of glufosinate on MON 88701 will follow the existing glufosinate-tolerant cotton uses outlined on the glufosinate herbicide label. Furthermore, glufosinate residues in MON 88701 treated with glufosinate are below the EPA-established residue tolerances of 4.0 ppm and 15.0 ppm for cottonseed and gin by-products, respectively (U.S. EPA, 2003) (40 CFR § 180.473). Both of these tolerances include the combined residues of parent glufosinate and its metabolites N-acetyl glufosinate and 3-methylphosphinico-propionic acid. Currently glufosinate is undergoing reregistration at EPA with the Reregistration Eligibility Decision (RED) expected by the end of 2013 (U.S. EPA, 2008). It is likely that EPA will affirm the safety and efficacy of glufosinate and approve its continued use in the marketplace upon completion of the reregistration process. Therefore, Monsanto will not pursue any changes in the glufosinate label, use pattern, or the established tolerances for its use on MON 88701 cotton.

I.C.3. Submissions to Foreign Government Agencies

To support commercial introduction of MON 88701 in the U.S., regulatory submissions will be made to countries that import significant quantities of cotton or its processed fractions from the U.S. These will include submissions to a number of foreign government regulatory authorities, including: Japan's Ministry of Agriculture, Forestry, and Fisheries and the Ministry of Health, Labour, and Welfare; the Canadian Food Inspection Agency; Health Canada; the Intersectoral Commission for Biosafety of Genetically Modified Organisms, Mexico; the Korea Food and Drug Administration; and the Rural Development Administration of Korea, as well as to regulatory authorities in other cotton importing countries with functioning regulatory systems. As appropriate, notifications will be made to countries that import significant quantities of cotton and cotton products that do not have a formal regulatory review process for biotechnology-derived crops.

II. THE BIOLOGY OF COTTON

The Organisation for Economic Co-operation and Development Consensus Document (OECD, 2008) on the biology of cotton (*Gossypium* spp.) provides key information on:

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

Additional information on the biology and growth and development of cotton is available in the literature (Kohel and Lewis, 1984; OGTR, 2008; Smith and Cothren, 1999).

To support the evaluation of the plant pest potential of MON 88701 relative to conventional cotton, additional information regarding several aspects of cotton biology can be found elsewhere in this petition. This includes: agronomic practices for cotton in Section V.III; volunteer management of cotton in Sections VIII.H and IX.C; and inter-species/genus introgression potential in Section IX.D.

II.A. Cotton as a Crop

Cotton belongs to the genus *Gossypium* that currently has approximately 50 species which are widely cultivated in tropical and subtropical regions around the world (OECD, 2008; Percival et al., 1999). There are four cultivated species that were domesticated independently, two of which account for greater than 95% of world cotton production. *Gossypium hirsutum* (often called upland, American, Mexican, or Acala cotton) accounts for 90% and *Gossypium barbadense* (often called extra long-staple, Pima, and Egyptian cotton) accounts for 5% of world cotton production. Due to the utility of the fibers for the production of textiles, human selection pressure on cotton has altered the plant from essentially perennial shrubs or trees with small impermeable seeds and sparse hairs to a compact annual row crop, yielding large, easily germinating seeds with white, thick, long, and strong fibers (Brubaker et al., 1999).

The four cultivated species, which are widely cultivated across the entire globe, are comprised of two diploid species *G. arboretum* and *G. herbaceum*, which evolved from Africa and the Middle East, and two allotetraploid species *G. barbadense* and *G. hirsutum*, which evolved in the Americas (Brubaker et al., 1999).

Improved modern varieties of *G. hirsutum* and *G. barbadense* are currently cultivated in the southern U.S., with *G. barbadense* grown primarily in the western states of Arizona, California, New Mexico, and Texas; and *G. hirsutum* produced throughout the 17 states comprising the U.S. cotton growing region, commonly referred to as the cotton belt. *G. hirsutum* comprises the vast majority of U.S. cotton production with nearly 11 million

acres planted and 18 million bales harvested, whereas *G. barbadense* varieties accounted for approximately 200,000 acres and half a million bales in 2010 (USDA-NASS, 2011e). Commercial cotton, including *G. hirsutum* and *G. barbadense*, has a long history of agricultural production (Lee, 1984; USDA-AMS, 2001; USDA-NASS, 2012c). Extra-long staple lint from *G. barbadense* is segregated and classed separately from *G. hirsutum* and is sold at a premium (USDA-AMS, 2001). However, cottonseed and cottonseed by-products (*e.g.*, oil and meal) are not generally distinguished by species (OECD, 2008; USDA-FAS, 2005).

II.B. Characteristics of the Recipient Plant

The *G. hirsutum* cotton variety used as the recipient for the DNA insertion to create MON 88701 was Coker 130, a non-transgenic, conventional, upland inbred variety developed by Coker Pedigreed Seed Co., commercialized in 1990 in the U.S. (Bowman et al., 2006).

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

Coker 130 was used as the near isogenic, conventional parental cotton comparator (referred to in this petition as the conventional control) in the safety assessment of MON 88701. MON 88701 and the conventional control have similar genetic backgrounds with the exception of the T-DNA, thus, the effect of the T-DNA and the expressed MON 88701 DMO and PAT (*bar*) proteins could be assessed. In addition, commercial cotton varieties (referred to in this consultation document as commercial reference varieties) were used as reference materials to establish ranges of natural variability representative of commercial cotton varieties. The commercial reference varieties used at each field trial location were selected based on their availability and agronomic fit for the respective geographic region.

III. DESCRIPTION OF THE GENETIC MODIFICATION

MON 88701 was developed through *Agrobacterium tumefaciens*-mediated transformation of cotton tissues from Coker 130 variety utilizing plasmid vector PV-GHHT6997. This section describes the plasmid vector, the donor gene, and the regulatory elements used in the development of MON 88701, as well as the deduced amino acid sequence of the MON 88701 DMO protein and PAT (*bar*) protein produced in MON 88701. In this section, transfer DNA (T-DNA) refers to DNA that is transferred to the plant during transformation. An expression cassette is comprised of sequences to be transcribed and the regulatory elements necessary for the expression of those sequences.

III.A. PV-GHHT6997

PV–GHHT6997 was used in the transformation of cotton to produce MON 88701 and its plasmid map is shown in Figure III-1. The elements included in this plasmid vector are described in Table III-1. PV- GHHT6997 is approximately 9.4 kb and contains one T-DNA that is delineated by Left Border and Right Border regions. The T-DNA contains the *dmo* and *bar* expression cassettes. The *dmo* expression cassette is regulated by the peanut chlorotic streak caulimovirus (*PCISV*) promoter, the tobacco etch virus (*TEV*) 5′ leader sequence, and the 3′ untranslated sequence of the *E6* gene from *Gossypium barbadense*. The chloroplast transit peptide CTP2 directs transport of the DMO protein to the chloroplast in MON 88701 and is derived from the *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene (Herrmann, 1995; Klee et al., 1987). The *bar* expression cassette is regulated by the *e35S* promoter from the 35S RNA of cauliflower mosaic virus (CaMV), the heat shock protein 70 (*Hsp70)* leader, and the nopaline synthase (*nos)* 3′ untranslated region.

The backbone region of PV–GHHT6997, located outside of the T-DNA, contains two origins of replication for maintenance of plasmid vector in bacteria (*oriV* and *ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer (*rop*) protein for maintenance of plasmid vector copy number in *Escherichia coli* (*E. coli*). A description of the genetic elements and their prefixes (*e.g.*, B-, P-, L-, TS-, CS-, T-, and OR-) in PV-GHHT6997 is provided in Table III-1.

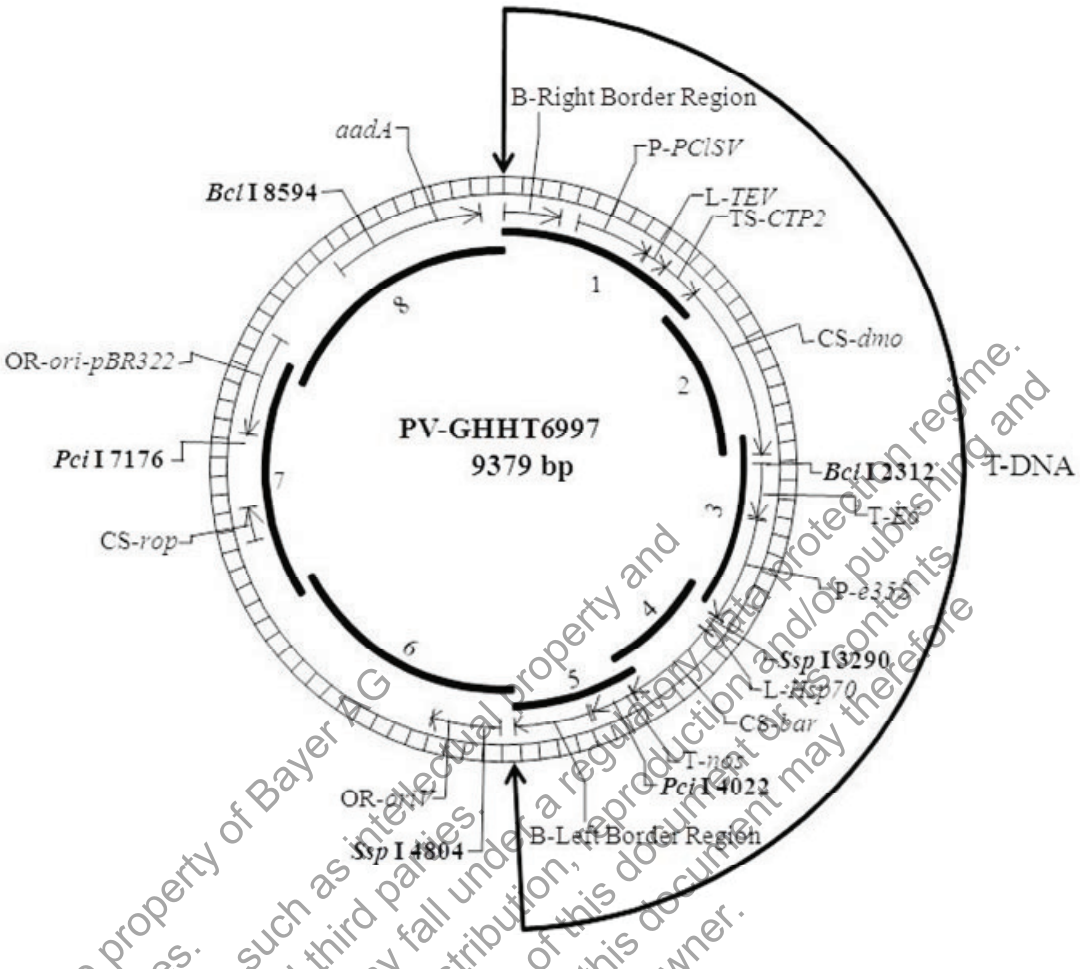
III.B. Description of the Transformation System

MON 88701 was developed through *Agrobacterium*-mediated transformation of PV-GHHT6997 (Figure III-1) into cotton hypocotyls, based on published methods ([redacted], 2010; [redacted] and [redacted], 2011). In summary, hypocotyl segments were excised from dark grown seedlings of germinated Coker 130 seed. After co-culturing with the *Agrobacterium*[3] carrying the vector, the hypocotyl segments were placed on a sequence of media for callus growth containing carbenicillin and cefotaxime to inhibit the growth of excess *Agrobacterium* and glufosinate to inhibit growth of untransformed cells. The

[3] *Agrobacterium* strain used contained a disarmed Ti plasmid.

somatic embryos developing on the culture medium were then placed on medium that contained plant growth regulators conducive to shoot regeneration, but no antibiotics or glufosinate. Rooted plants (R_0) with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

The R_0 plants generated through the *Agrobacterium*-mediated transformation were self-pollinated to produce R_1 seed. R_0 and R_1 plants were evaluated for tolerance to dicamba and glufosinate and screened for the presence of the T-DNA (*dmo* and *bar* expression cassettes) and absence of plasmid vector backbone (o*riV*). Subsequently, the *dmo* and *bar* homozygous positive R_1 plant was self-pollinated to give rise to R_2 plants. Homozygous positive R_2 plants containing only a single T-DNA insertion, were identified by a combination of analytical techniques including dicamba and glufosinate sprays, polymerase chain reaction (PCR), and Southern blot analysis, resulting in production of dicamba and glufosinate-tolerant cotton MON 88701. MON 88701 was selected as the lead event based on superior phenotypic characteristics and its molecular characteristics. Studies on MON 88701 were initiated to further characterize the genetic insertion and the expressed proteins, and to establish the food, feed, and environmental safety relative to conventional cotton. The major steps involved in the development of MON 88701 are depicted in Figure III-2.



Probe	Start Position (bp)	End Position (bp)	Total Length (~kb)
1	1	1310	1.3
2	1223	2241	1.0
3	2142	3252	1.1
4	3153	3914	0.8
5	3832	4625	0.8
6	4626	6282	1.7
7	6204	7708	1.5
8	7630	9379	1.8

Figure III-1. Circular Map of PV-GHHT6997 Showing Probes 1-8

A circular map of PV-GHHT6997 used to develop MON 88701 is shown. PV-GHHT6997 contains a single T-DNA. Genetic elements and restriction sites (in bold) used in Southern analyses (with positions relative to the first base pair of the plasmid vector) are shown on the exterior of the map. The probes used in the Southern analyses are shown on the interior of the map and listed in the table.

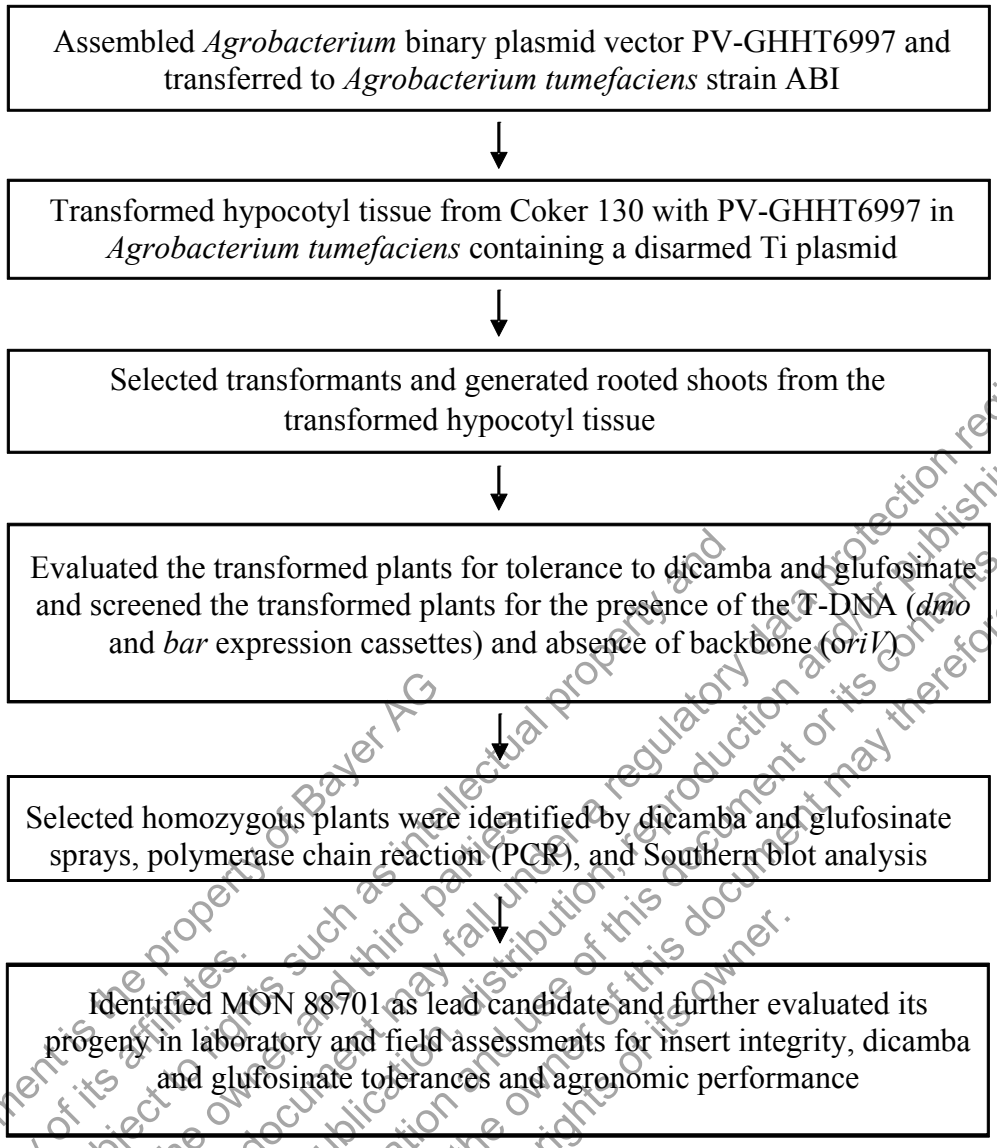


Figure III-2. Schematic of the Development of MON 88701

III.C. The *dmo* Coding Sequence and the MON 88701 DMO Protein

The *dmo* expression cassette encodes a ~39 kDa MON 88701 DMO precursor protein consisting of a single polypeptide of 416 amino acids (Figure III-3). The *dmo* coding sequence is the codon optimized coding sequence from *Stenotrophomonas maltophilia* that encodes the DMO protein (Herman et al., 2005; Wang et al., 1997). The presence of MON 88701 DMO protein confers dicamba tolerance.

III.D. The *bar* Coding Sequence and PAT (*bar*) Protein

The *bar* expression cassette encodes a ~21 kDa PAT (*bar*) protein consisting of a single polypeptide of 183 amino acids ([redacted] et al., 1987) (Figure III-4). The *bar* coding sequence is from *Streptomyces hygroscopicus* and encodes the phosphinothricin N-acetyltransferase (PAT) protein ([redacted] et al., 1987). The presence of PAT (*bar*) protein confers glufosinate tolerance.

III.E. Regulatory Sequences

The *dmo* coding sequence in MON 88701 is under the regulation of the *PC1SV* promoter, the *TEV* 5′ leader, and the *E6* 3′ untranslated region. The *PC1SV* promoter is the promoter for the Full-Length Transcript (FLt) of peanut chlorotic streak caulimovirus (Maiti and Shepherd, 1998) that directs transcription in plant cells. The *TEV* leader is the 5′ untranslated region from the tobacco etch virus (Niepel and Gallie, 1999) and is involved in regulating gene expression. The chloroplast transit peptide CTP2 directs transport of the DMO protein to the chloroplast in MON 88701 and is derived from the *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene (Herrmann, 1995; Klee et al., 1987). The *E6* 3′ non-translated region is the 3′ untranslated region from the *E6* gene of *Gossypium barbadense* encoding a fiber protein, which functions to direct polyadenylation of the mRNA (John, 1996).

The *bar* coding sequence in MON 88701 is under the regulation of the *e35S* promoter, the *Hsp70* leader, and the *nos* 3′ untranslated region. The *e35S* promoter is the promoter for the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985), containing the duplicated enhancer region (Kay et al., 1987) that directs transcription in plant cells. The *Hsp70* leader is the 5′ untranslated region from the *DnaK* gene from *Petunia hybrida* (Rensing and Maier, 1994; Winter et al., 1988) and is involved in regulating gene expression. The *nos* 3′ untranslated region is the 3′ untranslated region from the nopaline synthase (*nos*) gene of *Agrobacterium tumefaciens* encoding NOS and directs polyadenylation of the mRNA (Bevan et al., 1983; Fraley et al., 1983).

III.F. T-DNA Borders

PV-GHHT6997 contains Right Border and Left Border regions (Figure III-1 and Table III-1), which were derived from *Agrobacterium tumefaciens* plasmids. The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation (Barker et al., 1983; Depicker et al., 1982; Zambryski et al., 1982). The border regions separate the T-DNA from the plasmid backbone region and are involved in the efficient transfer of T-DNA into the cotton genome.

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

Genetic elements that exist outside of the T-DNA border regions are those that are essential for the maintenance or selection of PV-GHHT6997 in bacteria. The origin of replication, *oriV,* is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host plasmid RK2 (Stalker et al., 1981). The origin of replication, *ori-pBR322,* is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid vector pBR322 (Sutcliffe, 1979). Coding sequence *rop* encodes the repressor of primer (ROP) protein which is necessary for the maintenance of plasmid 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 cotton genome. The absence of detectable backbone sequence in MON 88701 has been confirmed by Southern blot analyses (See Section IV–B).

Table III-1. Summary of Genetic Elements in PV-GHHT6997

Genetic Element	Location in Plasmid Vector (bp)	Function (Reference)
T-DNA		
B[1]-Right Border Region	1-331	DNA region from *Agrobacterium tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)
Intervening Sequence	332-433	Sequence used in DNA cloning
P[2]-*PC1SV*	434-866	Promoter from the Full-Length Transcript (FLt) of peanut chlorotic streak caulimovirus (*PC1SV*) that directs transcription in plant cells (Maiti and Shepherd, 1998)
Intervening Sequence	867-872	Sequence used in DNA cloning
L[3]-*TEV*	873-1004	5′ UTR leader sequence from the RNA of tobacco etch virus (TEV) (Niepel and Gallie, 1999) that is involved in regulating gene expression
Intervening Sequence	1005	Sequence used in DNA cloning
TS[4]-*CTP2*	1006-1233	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann, 1995; Klee et al., 1987)
CS[5]-*dmo*	1234-2256	Codon optimized coding sequence for the dicamba mono-oxygenase (DMO) protein of *Stenotrophomonas maltophilia* that confers dicamba tolerance (Herman et al., 2005; Wang et al., 1997)
Intervening Sequence	2257-2310	Sequence used in DNA cloning
T[6]-*E6*	2311-2625	3′ UTR sequence of the *E6* gene from *Gossypium barbadense* (cotton) encoding a fiber protein involved in early fiber development (John, 1996) that directs polyadenylation of mRNA
Intervening Sequence	2626-2637	Sequence used in DNA cloning
P-*e35S*	2638-3249	Promoter from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) containing the duplicated enhancer region (Kay et al., 1987) that directs transcription in plant cells
Intervening Sequence	3250-3252	Sequence used in DNA cloning

Table III-1. Summary of Genetic Elements in PV-GHHT6997 (continued)

Genetic Element	Location in Plasmid Vector (bp)	Function (Reference)
L-*Hsp70*	3253-3348	5′ UTR leader sequence of the *DnaK* gene from *Petunia hybrida* that encodes heat shock protein 70 (HSP70) (Rensing and Maier, 1994; Winter et al., 1988) that is involved in regulating gene expression
Intervening Sequence	3349-3354	Sequence used in DNA cloning
CS-*bar*	3355-3906	Coding sequence for the phosphinothricin N-acetyltransferase (PAT) protein of *Streptomyces hygroscopicus* that confers glufosinate tolerance ([redacted] et al., 1987)
Intervening Sequence	3907-3911	Sequence used in DNA cloning
T-*nos*	3912-4164	3′ UTR sequence of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* pTi encoding NOS that directs polyadenylation (Bevan et al., 1983; Fraley et al., 1983)
Intervening Sequence	4165-4183	Sequence used in DNA cloning
B-Left Border Region	4184-4625	DNA region from *Agrobacterium tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983)
		Plasmid Vector Backbone
Intervening Sequence	4626-4711	Sequence used in DNA cloning
OR[7]-*oriV*	4712-5108	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	5109-6616	Sequence used in DNA cloning
CS-*rop*	6617-6808	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	6809-7235	Sequence used in DNA cloning
OR-*ori-pBR322*	7236-7824	Origin of replication from plasmid pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)

Table III-1. Summary of Genetic Elements in PV-GHHT6997 (continued)

Genetic Element	Location in Plasmid Vector (bp)	Function (Reference)
Intervening Sequence	7825-8354	Sequence used in DNA cloning
aadA	8355-9243	Bacterial 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	9244-9379	Sequence used in DNA cloning

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

```
  1   MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTQQHP RAYPISSSWG
 51   LKKSGMTLIG SELRPLKVMS SVSTACMLTF VRNAWYVAAL PEELSEKPLG
101   RTILDTPLAL YRQPDGVVAA LLDICPHRFA PLSDGILVNG HLQCPYHGLE
151   FDGGGQCVHN PHGNGARPAS LNVRSFPVVE RDALIWIWPG DPALADPGAI
201   PDFGCRVDPA YRTVGGYGHV DCNYKLLVDN LMDLGHAQYV HRANAQTDAF
251   DRLEREVIVG DGEIQALMKI PGGTPSVLMA KFLRGANTPV DAWNDIRWNK
301   VSAMLNFIAV APEGTPKEQS IHSRGTHILT PETEASCHYF FGSSRNFGID
351   DPEMDGVLRS WQAQALVKED KVVVEAIERR RAYVEANGIR PAMLSCDEAA
401   VRVSREIEKL EQLEAA
```

Figure III-3. Deduced Amino Acid Sequence of the MON 88701 DMO Protein
The amino acid sequence of the MON 88701 DMO precursor protein was deduced from the full-length coding nucleotide sequence present in PV-GHHT6997 (See Table III-1 for more detail). The chloroplast transit peptide (CTP2) and the first 76 amino acids of the precursor protein are underlined. CTP2 targets MON 88701 DMO protein to the chloroplast. The CTP2 is cleaved in the chloroplast producing the mature 349 amino acid MON 88701 DMO protein that begins with the valine at position 68 (See Appendix C.1). The double underline shows the nine amino acids from CTP2 that are at the N-terminus of the mature MON 88701 protein.

```
  1   MSPERRPADI RRATEADMPA VCTIVNHYIE TSTVNFRTEP QEPQEWTDDL
 51   VRLRERYPWL VAEVDGEVAG IAYAGPWKAR NAYDWTAEST VYVSPRHQRT
101   GLGSTLYTHL LKSLEAQGFK SVVAVIGLPN DPSVRMHEAL GYAPRGMLRA
151   AGFKHGNWHD VGFWQLDFSL PVPPRPVLPV TEI
```

Figure III-4. Deduced Amino Acid Sequence of the PAT (*bar*) Protein
The amino acid sequence of the MON 88701-produced PAT (*bar*) protein was deduced from the full-length coding nucleotide sequence present in PV-GHHT6997 (See Table III-1 for more detail).

IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

Characterization of the DNA insert in MON 88701 was conducted by Southern blot, PCR, and DNA sequence analyses. The results of this characterization demonstrate that MON 88701 contains a single copy of the *dmo* and *bar* expression cassettes and lacks plasmid backbone; the T-DNA is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These conclusions were based on several lines of evidence: 1) Southern blot analyses assayed the entire cotton genome for the presence of the T-DNA and absence of the plasmid backbone sequences derived from PV-GHHT6997, and demonstrated that only a single copy of the T-DNA was inserted at a single genomic site and that the insert is stably inherited; 2) DNA sequence analyses to determine the exact sequence of the inserted DNA and the DNA sequences flanking the 5′ and 3′ ends of the insert, allowing a comparison to the T-DNA sequence in the plasmid vector to confirm that only the expected sequences were integrated; 3) DNA sequences flanking the 5′ and 3′ ends of the insert were compared to the sequence of the insertion site in conventional cotton 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 stably integrated at a single locus of the cotton genome and that no plasmid backbone sequences are present in MON 88701.

Southern blot analyses were used to determine the copy number and insertion sites of the integrated DNA as well as the presence or absence of plasmid vector backbone sequences. The Southern blot strategy was designed to ensure that all potential transgenic segments would be identified. The entire cotton genome was assayed with probes that spanned the complete plasmid vector to detect the presence of the insert as well as confirm the absence of any plasmid vector backbone sequences. This was accomplished by using probes that were not more than 2.5 kb in length to ensure a high level of sensitivity. This high level of sensitivity was demonstrated for each blot by detection of a positive control added at 0.1 copies per genome equivalent. Two sets of restriction enzymes were specifically chosen to fully characterize the T-DNA and detect any potential fragments of the T-DNA and backbone sequences. The restriction enzyme sets were chosen such that each enzyme set cleaves once within the inserted T-DNA and at least once within the known DNA flanking the 5′ or 3′ end of the insert. As a consequence, at least one segment containing a portion of the insert with the adjacent 5′ flanking DNA generated by one set of the enzyme(s) is of a predictable size and overlaps with another predictable size segment containing a portion of the insert with the adjacent 3′ flanking DNA generated by another set of the enzyme(s). This two-set enzyme design ensures that the entire insert is identified in a predictable hybridization pattern. This strategy also maximizes the possibility of detecting an insertion elsewhere in the genome that could be overlooked if that band co-migrated on the gel with an expected band.

To determine the number of copies and insertion sites of the T-DNA, and the presence or absence of the plasmid vector backbone sequences, duplicated samples that consisted of equal amounts of digested DNA were run on the agarose gel. One set of samples was run for a longer period of time (long run) than the second set (short run). The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows for

retaining the small molecular weight DNA on the gel. The molecular weight markers on the left of the figures were used to estimate the sizes of the bands present in the long run lanes of the Southern blots, and the molecular weight markers on the right of the figures were used to estimate the sizes of bands present in the short run lanes of the Southern blots (Figure IV-2 through Figure IV-5). Any minor discrepancies between the molecular weight marker and the genomic DNA samples are likely due to differences in the migration rate of DNA during agarose gel electrophoresis caused by differences in salt concentration, base composition, or sequences of DNA (Elder and Southern, 1983; Sambrook and Russell, 2001). Southern blot analyses determined that a single copy of the T-DNA was inserted at a single locus of the cotton genome, and no additional genetic elements, including backbone sequences, from PV-GHHT6997 were detected in MON 88701.

The PCR and DNA sequence analyses complement the Southern analyses. PCR and DNA sequence analyses performed on MON 88701 determined the complete DNA sequence of the insert and flanking genomic DNA sequences in MON 88701, confirmed the predicted organization of the genetic elements within the insert, and determined the sequences flanking the insert. In addition, DNA sequence analyses confirmed that each genetic element (except for the border regions) in the insert is intact and the sequence of the insert is identical to the corresponding sequence in PV-GHHT6997 (Figures IV-6 and IV-7). Furthermore, genomic organization at the MON 88701 insertion site was determined by comparing the sequence flanking the 5′ and 3′ ends of the insert to the sequence of the insertion site in conventional cotton.

The stability of the T-DNA present in MON 88701 across multiple generations was demonstrated by Southern blot fingerprint analysis (Figure IV-9). Genomic DNA from five generations of MON 88701 (Figure IV-8) was digested with one of the enzyme sets used for the insert and copy number analyses and was hybridized with two probes that detect restriction segments that encompass the entire insert. This fingerprint strategy consists of two insert segments each containing its adjacent genomic DNA that assesses not only the stability of the insert, but also the stability of the DNA directly adjacent to the insert.

Segregation analysis was conducted to determine the inheritance and stability of the T-DNA insert in MON 88701. Results from this analysis demonstrated that the inheritance and stability of the insert was as expected across multiple generations (Figure IV-8, Table IV-3, and Table IV-4), which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA at a single chromosomal locus.

The Southern blot analyses confirmed that the T-DNA reported in Figure IV-1 represents the only detectable insert in MON 88701. A circular map of PV-GHHT6997 annotated with the probes used in the Southern blot analysis is presented in Figure III-1 and the genetic elements within the MON 88701 insert are summarized in Table IV-2. A linear map depicting restriction sites within the insert as well as within the DNA immediately flanking the insert in MON 88701 is shown in Figure IV-1. Based on the plasmid map and the linear map of the insert, a table summarizing the expected DNA segments for

Southern analyses is presented in Table IV-1. The results from the Southern blot analyses are presented in Figure IV-2 through Figure IV-5. PCR amplification of the MON 88701 insert and the insertion site in the conventional control for DNA sequence analysis are shown in Figure IV-6 and Figure IV-7, respectively. The generations used in the generational stability analysis are depicted in the breeding history shown in Figure IV-8 and the results from the generational stability analysis are presented in Figure IV-9. The breeding path for generating the segregation data is shown in Figure IV-10 and the results for the segregation analysis are presented in Table IV-3 and IV-4. Materials and methods used for the characterization of the insert in MON 88701 are found in Appendix B.

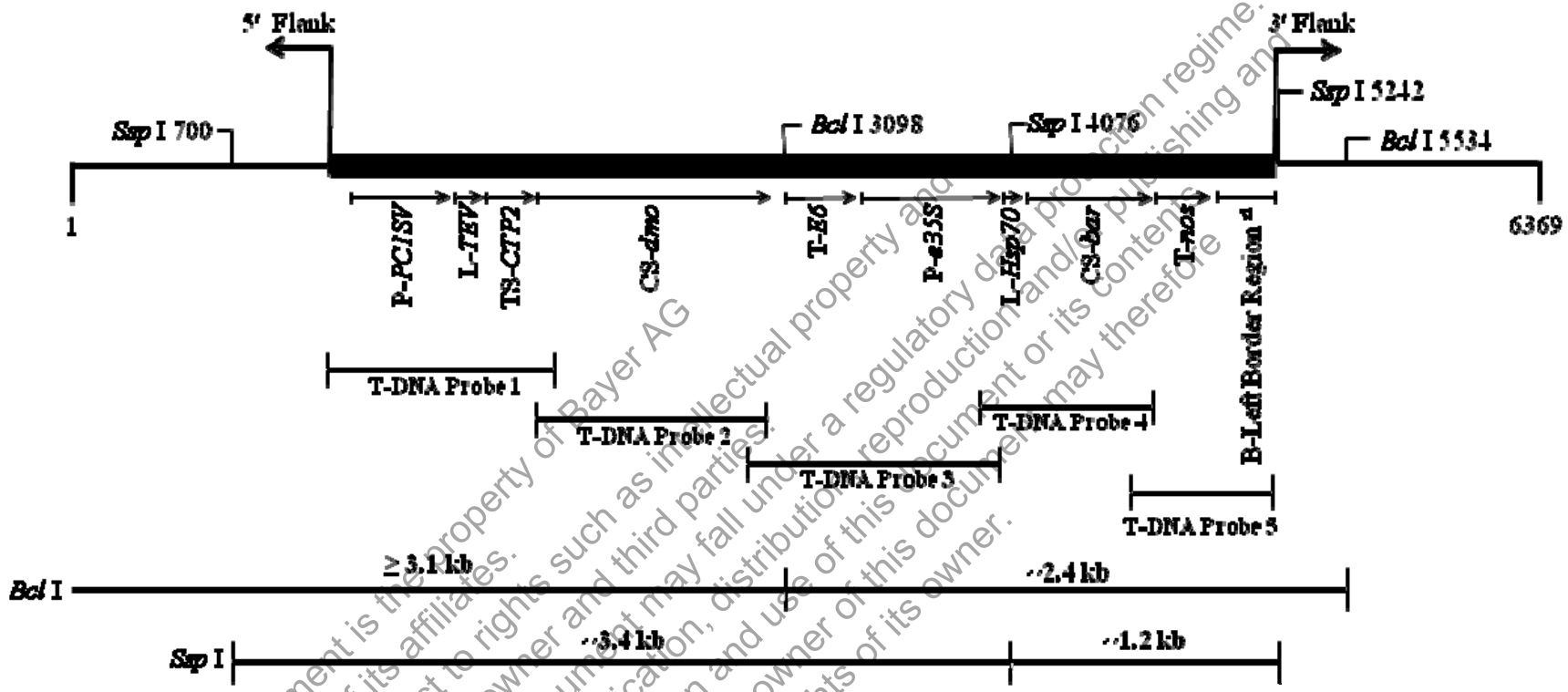


Figure IV-1. Schematic Representation of the Insert and Flanking DNA in MON 88701

A linear map of the insert and DNA flanking the insert in MON 88701 is shown. Angled arrows indicate the ends of the integrated T-DNA and the beginning of the flanking DNA. Identified on the linear map are genetic elements within the insert, as well as the sites of the restriction enzymes used in the Southern analyses with positions relative to the first base pair of the DNA sequence represented in this map. The relative sizes and locations of the T-DNA probes and the expected sizes of restriction fragments are indicated in the lower portion of the scheme. This schematic diagram is not drawn to scale. Locations of genetic elements and T-DNA probes are approximate. Probes are also shown in Figure III-1. [r1]Superscript in Left Border Region indicates that the sequence in MON 88701 was truncated compared to the sequences in PV–GHHT6997.

Table IV-1. Summary Chart of the Expected DNA Segments Based on Hybridizing Probes and Restriction Enzymes Used in MON 88701 Analysis

Southern Blot Analysis		T-DNA			Backbone
Figure		**IV-2**	**IV-3**	**IV-4**	**IV-5**
Probe(s) Used		1,5	2,4	3	6, 7, 8
Probing Target	**Digestion enzyme**	**Expected Band Sizes on each Southern Blot**			
PV-GHHT6997	*Pci* I	~6.2 kb ~3.2 kb	~6.2 kb	~6.2 kb	~6.2 kb ~3.2 kb
Probe Templates[1]	N/A	~1.3 kb ~0.8 kb	~1.0 kb ~0.8 kb	~~[2]	~1.5 kb ~1.7 kb ~1.8 kb
MON 88701	*Bcl* I	≥3.1 kb ~2.4 kb	≥3.1 kb ~2.4 kb	≥3.1 kb ~2.4 kb	None
	Ssp I	~3.4 kb ~1.2 kb	~3.4 kb ~1.2 kb	~3.4 kb	None

[1]Probe template spikes were used as positive hybridization controls in Southern blot analyses when multiple probes were hybridized to the blot simultaneously.
[2]' ~~' indicates that probe template was not used.

Table IV-2. Summary of Genetic Elements in MON 88701

Genetic Element	Location in Sequence (bp)	Function (Reference)
5′ Flank	1-1126	Cotton genomic DNA
Intervening Sequence	1127-1219	Sequence used in DNA cloning
P[1]-*PC1SV*	1220-1652	Promoter from the Full-Length Transcript (FLt) of peanut chlorotic streak caulimovirus (*PC1SV*) that directs transcription in plant cells (Maiti and Shepherd, 1998)
Intervening Sequence	1653-1658	Sequence used in DNA cloning
L[2]-*TEV*	1659-1790	5′ UTR leader sequence from the RNA of tobacco etch virus (TEV) (Niepel and Gallie, 1999) that is involved in regulating gene expression
Intervening Sequence	1791	Sequence used in DNA cloning
TS[3]-*CTP2*	1792-2019	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann, 1995; Klee et al., 1987)
CS[4]-*dmo*	2020-3042	Codon optimized coding sequence for the dicamba mono-oxygenase (DMO) protein of *Stenotrophomonas maltophilia* that confers dicamba tolerance ([redacted] et al., 2005; [redacted] et al., 1997)
Intervening Sequence	3043-3096	Sequence used in DNA cloning
T[5]-*E6*	3097-3411	3′ UTR sequence of the *E6* gene from *Gossypium barbadense* (cotton) encoding a fiber protein involved in early fiber development (John, 1996) that directs polyadenylation of mRNA
Intervening Sequence	3412-3423	Sequence used in DNA cloning
P-*e35S*	3424-4035	Promoter from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) containing the duplicated enhancer region (Kay et al., 1987) that directs transcription in plant cells

Table IV-2. Summary of Genetic Elements in MON 88701 (continued)

Genetic Element	Location in Sequence (bp)	Function (Reference)
Intervening Sequence	4036-4038	Sequence used in DNA cloning
L-*Hsp70*	4039-4134	5′ UTR leader sequence of the *DnaK* gene from *Petunia hybrida* that encodes heat shock protein 70 (HSP70) (Rensing and Maier, 1994; Winter et al., 1988) that is involved in regulating gene expression
Intervening Sequence	4135-4140	Sequence used in DNA cloning
CS-*bar*	4141-4692	Coding sequence for the phosphinothricin N-acetyltransferase (PAT) protein of *Streptomyces hygroscopicus* that confers glufosinate tolerance ([redacted] et al., 1987)
Intervening Sequence	4693-4697	Sequence used in DNA cloning
T-*nos*	4698-4950	3′ UTR sequence of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* pTi encoding NOS that directs polyadenylation (Bevan et al., 1983; Fraley et al., 1983)
Intervening Sequence	4951-4969	Sequence used in DNA cloning
B[6]-Left Border Region[r1]	4970-5231	DNA region from *Agrobacterium tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983)
3′ Flank	5232-6369	Cotton genomic DNA

[1]P, Promoter
[2]L, Leader
[3]TS, Targeting Sequence
[4]CS, Coding Sequence
[5]T, Transcription Termination Sequence
[6]B, Border
[r1]Superscript in Left Border Region indicates that the sequence in MON 88701 was truncated compared to the sequences in PV-GHHT6997.

IV.A. Insert and Copy Number of T-DNA in MON 88701

The numbers of copies and insertion sites of the T-DNA sequences in the cotton genome were evaluated by digesting MON 88701 and conventional control genomic DNA samples with the restriction enzyme *Bcl* I or the restriction enzyme *Ssp* I and hybridizing Southern blots with probes that span the T-DNA (Figure III-1). Each restriction digest is expected to produce a specific banding pattern on the Southern blots (Table IV-1). Any additional copies and/or integration sites would be detected as additional bands on the blots.

The restriction enzyme *Bcl* I cleaves once within the inserted T-DNA and within the known genomic DNA flanking the 3′ end of the insert (Figure IV-1). Therefore, if T-DNA sequences were present as a single copy at a single integration site in MON 88701, the digestion with *Bcl* I was expected to generate two border segments with expected sizes of ≥3.1 kb and ~2.4 kb (Figure IV-1 and Table IV-1). The restriction enzyme *Ssp* I cleaves once within the inserted T-DNA and within the known genomic DNA flanking the 5′ and 3′ ends of the insert (Figure IV-1). If T-DNA sequences were present as a single copy at a single integration site in MON 88701, the digestion with *Ssp* I was expected to generate two border segments with expected sizes of ~3.4 kb and ~1.2 kb (Figure IV-1 and Table IV-1).

The Southern blots were hybridized with T-DNA probes that collectively span the entire inserted DNA sequence (Figures III-1 and IV-1, Probe 1, Probe 2, Probe 3, Probe 4, and Probe 5). Conventional control genomic DNA digested with the restriction enzyme *Bcl* I and spiked with either probe templates and/or digested PV-GHHT6997 DNA served as positive hybridization controls. The positive hybridization control was spiked at approximately 0.1 and 1.0 copies of genome equivalents to demonstrate sufficient sensitivity of the Southern blot. Conventional control genomic DNA digested with the appropriate restriction enzymes was used as a negative control. The results of these analyses are shown in Figure IV-2 through Figure IV-4.

IV.A.1. T-DNA Probes 1 and 5

Conventional control genomic DNA digested with *Bcl* I (Figure IV-2, Lane 1 and Lane 8) or with *Ssp* I (Figure IV-2, Lane 3 and Lane 10) and simultaneously hybridized with Probe 1 and Probe 5 (Figures III-1 and IV-1) produced no detectable hybridization bands as expected for the negative control in the reported exposure shown in Figure IV-2. In a longer exposure of the blot, faint endogenous hybridization bands were present in both the *Bcl* I digest and the *Ssp* I digest in the conventional control genomic DNA (data not shown). Conventional control genomic DNA digested with *Bcl* I and spiked with probe templates of Probe 1 and Probe 5 (Figure III-1) produced the expected bands at ~1.3 kb and ~0.8 kb (Figure IV-2, Lane 5 and Lane 6). Conventional control genomic DNA digested with *Bcl* I and spiked with the PV-GHHT6997 DNA, previously digested with the restriction enzyme *Pci* I (Figure III-1), produced two bands at ~6.2 kb and ~3.2 kb (Figure IV-2, Lane 7), as expected. Detection of the positive controls indicates that the probes hybridized to their target sequences.

MON 88701 DNA digested with *Bcl* I and simultaneously hybridized with Probe 1 and Probe 5 (Figures III-1 and IV-1) produced the expected bands at ~3.5 kb and ~2.4 kb (Figure IV-2, Lane 2 and Lane 9) which is consistent with the expected ≥3.1 kb and ~2.4 kb bands (Figure IV-1 and Table IV-1), respectively. MON 88701 DNA digested with the restriction enzyme *Ssp* I and hybridized with Probe 1 and Probe 5 (Figures III-1 and IV-1) produced two bands at ~3.4 kb and ~1.2 kb (Figure IV-2, Lane 4 and Lane 11), as expected.

The results presented in Figure IV-2 indicate that the sequences covered by Probe 1 and Probe 5 reside at a single detectable locus of integration in MON 88701.

IV.A.2. T-DNA Probes 2 and 4

Conventional control genomic DNA digested with *Bcl* I (Figure IV-3, Lane 1 and Lane 8) or with *Ssp* I (Figure IV-3, Lane 3 and Lane 10) and simultaneously hybridized with Probe 2 and Probe 4 (Figures III-1 and IV-1) produced no detectable hybridization bands as expected for the negative control. Conventional control genomic DNA digested with *Bcl* I and spiked with probe templates of Probe 2 and Probe 4 (Figure III-1) produced the expected bands at ~1.0 kb and ~0.8 kb (Figure IV-3, Lane 5 and Lane 6). Conventional control genomic DNA digested with *Bcl* I and spiked with the PV-GHHT6997 DNA, previously digested with the restriction enzyme *Pci* I (Figure III-1), produced one band at ~6.2 kb (Figure IV-3, Lane 7), as expected. Detection of the positive controls indicates that the probes hybridized to their target sequences.

MON 88701 DNA digested with *Bcl* I and simultaneously hybridized with Probe 2 and Probe 4 (Figures III-1 and IV-1) produced the expected bands at ~3.5 kb and ~2.4 kb (Figure IV-3, Lane 2 and Lane 9), which is consistent with the expected ≥3.1 kb and ~2.4 kb bands (Figure IV-1 and Table IV-1), respectively. MON 88701 DNA digested with the restriction enzyme *Ssp* I and hybridized with Probe 2 and Probe 4 (Figures III-1 and IV-1) produced two bands at ~3.4 kb and ~1.2 kb (Figure IV-3, Lane 4 and Lane 11), as expected.

The results presented in Figure IV-3 indicate that the sequences covered by Probe 2 and Probe 4 reside at a single detectable locus of integration in MON 88701.

IV.A.3. T-DNA Probe 3

Conventional control DNA digested with *Bcl* I (Figure IV-4, Lane 1 and Lane 7) or with *Ssp* I (Figure IV-4, Lane 3 and Lane 9) and hybridized with Probe 3 (Figures III-1 and IV-1) produced endogenous hybridization signals that were present in all lanes (Figure IV-4, Lane 1 through Lane 10). The same hybridization band was produced in conventional control and MON 88701 DNA lanes when digested with the same enzyme.

When digested with *Bcl* I and hybridized with Probe 3 hybridization bands of ~1.9 kb and ~1.7 kb were produced with conventional control genomic DNA and MON 88701 DNA (Figure IV-4, Lane 1, Lane 2, and Lane 5 through Lane 8). When digested with *Ssp* I and hybridized with Probe 3, a hybridization band of ~2.5 kb was produced with conventional control genomic DNA and MON 88701 DNA (Figure IV-4, Lane 3, Lane 4, Lane 9, and

Lane 10). Since these bands are present in both control and test substances, these signals are considered to be weak hybridization of probes to endogenous *E6* sequences and are not specific to the inserted DNA in MON 88701.

Conventional control genomic DNA digested with *Bcl* I and spiked with the PV-GHHT6997 DNA, previously digested with the restriction enzyme *Pci* I (Figure III-1), produced one band at ~6.2 kb (Figure IV-4, Lane 5 and Lane 6), as expected. Detection of the spiked controls indicates that the probe hybridized to its target sequence.

MON 88701 DNA digested with *Bcl* I and hybridized with Probe 3 (Figures III-1 and IV-1) produced two expected bands at ~3.5 kb and ~2.4 kb, which is consistent with the expected ≥3.1 kb and ~2.4 kb bands (Figure IV-1 and Table IV-1), and is in addition to the endogenous hybridization bands discussed above (Figure IV-4, Lane 2 and Lane 8). The ~3.5 kb band is less intense than the ~2.4 kb band. The difference in band intensity is likely due to hybridization of a smaller portion of Probe 3 to the ~3.5 kb fragment. The ~3.5 kb band represents the 5′ end of the inserted DNA and the adjacent DNA flanking the 5′ end of the insert; this correlates with the expected border fragment size of ≥3.1 kb. The ~2.4 kb band represents the 3′ end of the inserted DNA and the adjacent DNA flanking the 3′ end of the insert. MON 88701 DNA digested with *Ssp* I (Figure IV-4, Lane 4 and Lane 10, Figure IV-1, and Table IV-1) and hybridized with Probe 3 produced one expected band at ~3.4 kb in addition to the endogenous hybridization bands discussed above. The ~3.4 kb band represents the 5′ end of the inserted DNA and the adjacent DNA flanking the 5′ end of the insert.

The results presented in Figure IV-4 indicate that the sequence covered by Probe 3 resides at a single detectable locus of integration in MON 88701.

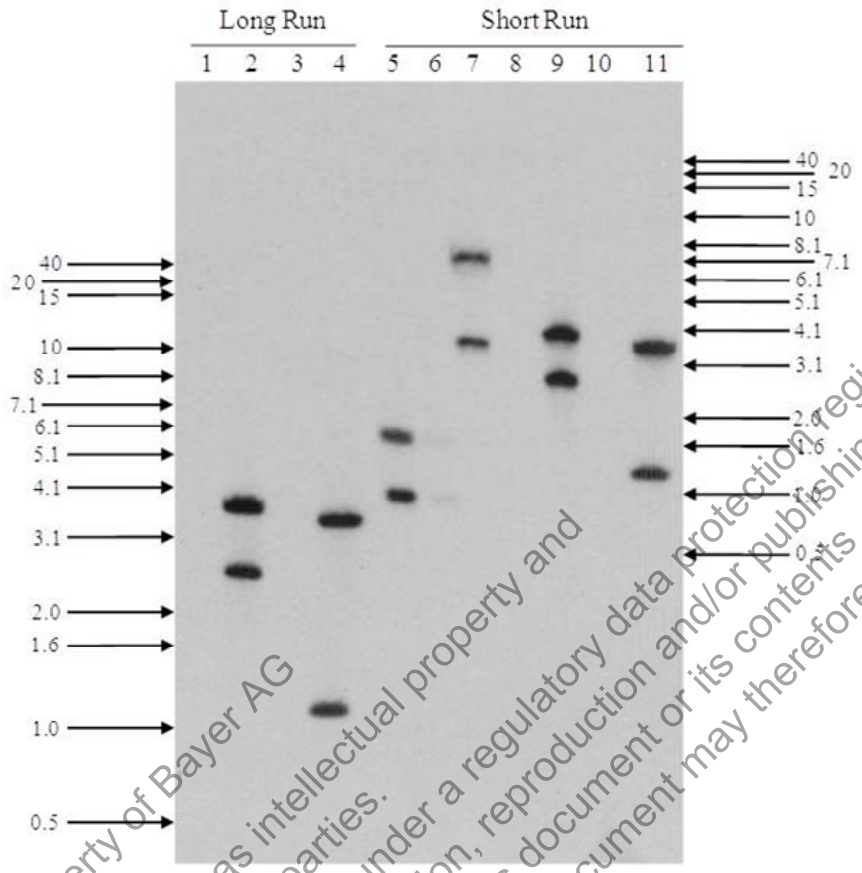


Figure IV-2. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA in MON 88701: Probes 1 and 5

The blot was simultaneously hybridized with two ^{32}P-labeled probes that span a portion of the T-DNA sequence (Figure III-1, Probe 1 and Probe 5). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Extension Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Bcl* I)
2	MON 88701 (*Bcl* I)
3	Conventional Control (*Ssp* I)
4	MON 88701 (*Ssp* I)
5	Conventional Control (*Bcl* I) spiked with Probe 1 and Probe 5 template [~1.0 genome equivalent]
6	Conventional Control (*Bcl* I) spiked with Probe 1 and Probe 5 template [~0.1 genome equivalent]
7	Conventional Control (*Bcl* I) spiked with PV-GHHT6997 (*Pci* I) [~1.0 genome equivalent]
8	Conventional Control (*Bcl* I)
9	MON 88701 (*Bcl* I)
10	Conventional Control (*Ssp* I)
11	MON 88701 (*Ssp* I)

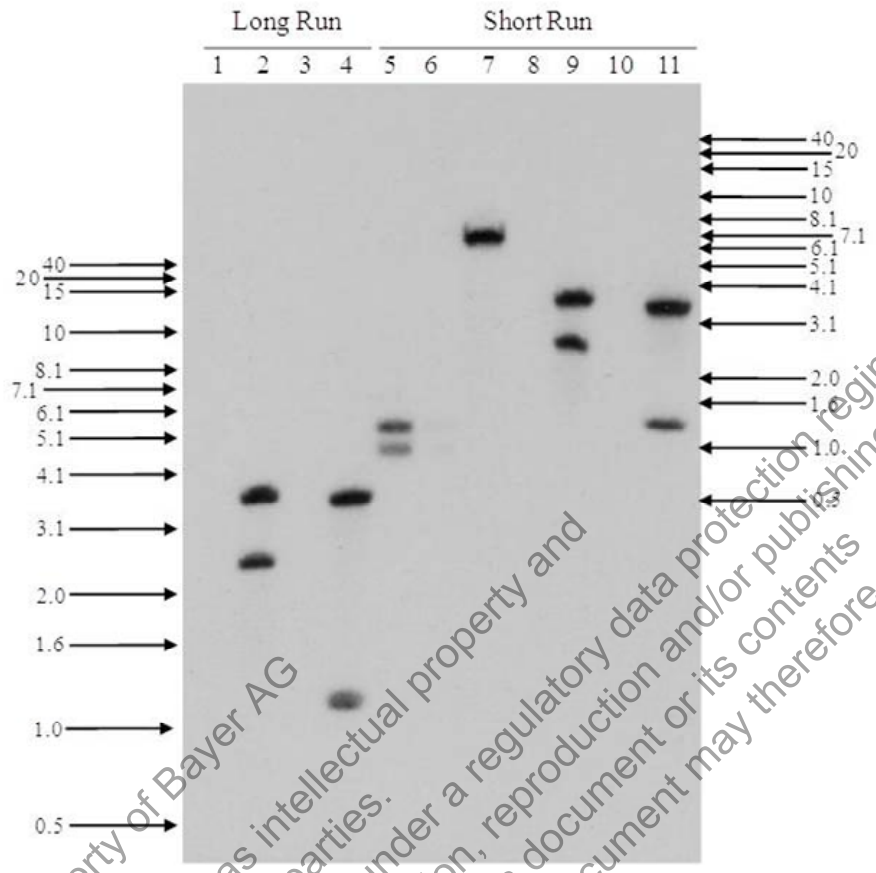


Figure IV-3. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA in MON 88701: Probes 2 and 4

The blot was simultaneously hybridized with two ^{32}P-labeled probes that span a portion of the T-DNA sequence (Figure III-1, Probe 2 and Probe 4). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Extension Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Bcl* I)
2	MON 88701 (*Bcl* I)
3	Conventional Control (*Ssp* I)
4	MON 88701 (*Ssp* I)
5	Conventional Control (*Bcl* I) spiked with Probe 2 and Probe 4 template [~1.0 genome equivalent]
6	Conventional Control (*Bcl* I) spiked with Probe 2 and Probe 4 template [~0.1 genome equivalent]
7	Conventional Control (*Bcl* I) spiked with PV-GHHT6997 (*Pci* I) [~1.0 genome equivalent]
8	Conventional Control (*Bcl* I)
9	MON 88701 (*Bcl* I)
10	Conventional Control (*Ssp* I)
11	MON 88701 (*Ssp* I)

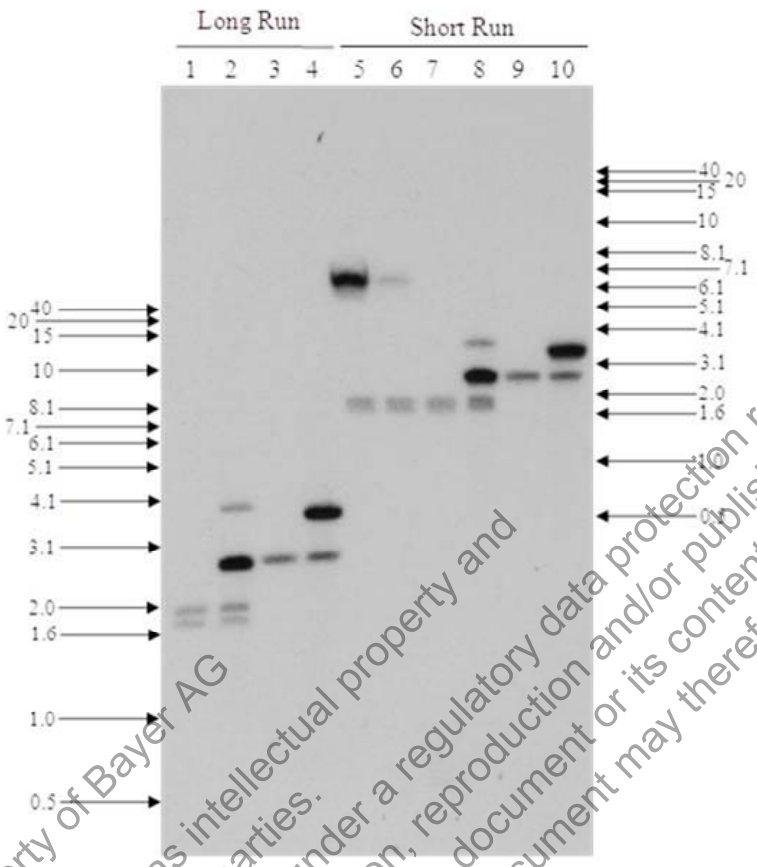


Figure IV-4. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA in MON 88701: Probe 3

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the T-DNA sequence (Figure III-1, Probe 3). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Extension Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	Conventional Control (*Bcl* I)
2	MON 88701 (*Bcl* I)
3	Conventional Control (*Ssp* I)
4	MON 88701 (*Ssp* I)
5	Conventional Control (*Bcl* I) spiked with PV–GHHT6997 (*Pci* I) [~1.0 genome equivalent]
6	Conventional Control (*Bcl* I) spiked with PV–GHHT6997 (*Pci* I) [~0.1 genome equivalent]
7	Conventional Control (*Bcl* I)
8	MON 88701 (*Bcl* I)
9	Conventional Control (*Ssp* I)
10	MON 88701 (*Ssp* I)

IV.B. Southern Blot Analysis to Determine the Presence or Absence of PV-GHHT6997 Backbone Sequences in MON 88701

To determine the presence or absence of the PV-GHHT6997 backbone sequences, MON 88701 and conventional control genomic DNA were digested with the restriction enzyme *Bcl* I or restriction enzyme *Ssp* I, and hybridized with the three backbone probes that collectively span the entire backbone sequences (Figure III-1, Probe 6, Probe 7, and Probe 8). If backbone sequences are present in MON 88701, then probing with backbone probes should result in hybridizing bands. Conventional control genomic DNA digested with the restriction enzyme *Bcl* I and spiked with probe templates or with digested PV-GHHT6997 DNA served as positive hybridization controls. The positive hybridization control was spiked at approximately 0.1 and 1.0 copies of genome equivalents to demonstrate sufficient sensitivity of the Southern blot. Conventional control genomic DNA digested with the appropriate restriction enzymes was used as a negative control. The results of these analyses are shown in Figure IV-5.

IV.B.1. Backbone Probes 6, 7, and 8

Conventional control DNA digested with *Bcl* I (Figure IV-5, Lane 1 and Lane 10) or the restriction enzyme *Ssp* I (Figure IV-5, Lane 3 and Lane 12) and hybridized with Probe 6, Probe 7, and Probe 8 (Figure III-1) produced no detectable hybridization bands as expected for the negative control.

Conventional control genomic DNA digested with *Bcl* I and spiked with probe templates of Probe 7 and Probe 8 (Figure III-1) produced the expected bands at ~1.5 kb and ~1.8 kb (Figure IV-5, Lane 5 and Lane 6). Conventional control genomic DNA digested with *Bcl* I and spiked with probe template of Probe 6 (Figure III-1) produced the one expected band at ~1.7 kb (Figure IV-5, Lane 7 and Lane 8). Conventional control DNA digested with *Bcl* I and spiked with the PV-GHHT6997 DNA, previously digested with the restriction enzyme *Pci* I (Figure III-1), produced two bands at ~6.2 kb and ~3.2 kb (Figure IV-5, Lane 9), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequence.

MON 88701 DNA digested with *Bcl* I (Figure IV-5, Lane 2 and Lane 11) or the restriction enzyme *Ssp* I (Figure IV-5, Lane 4 and Lane 13) and hybridized with Probes 6, 7, and 8 produced no detectable bands.

The results presented in Figure IV-5 indicate that MON 88701 contains no detectable backbone sequences covered by Probes 6, 7, and 8.

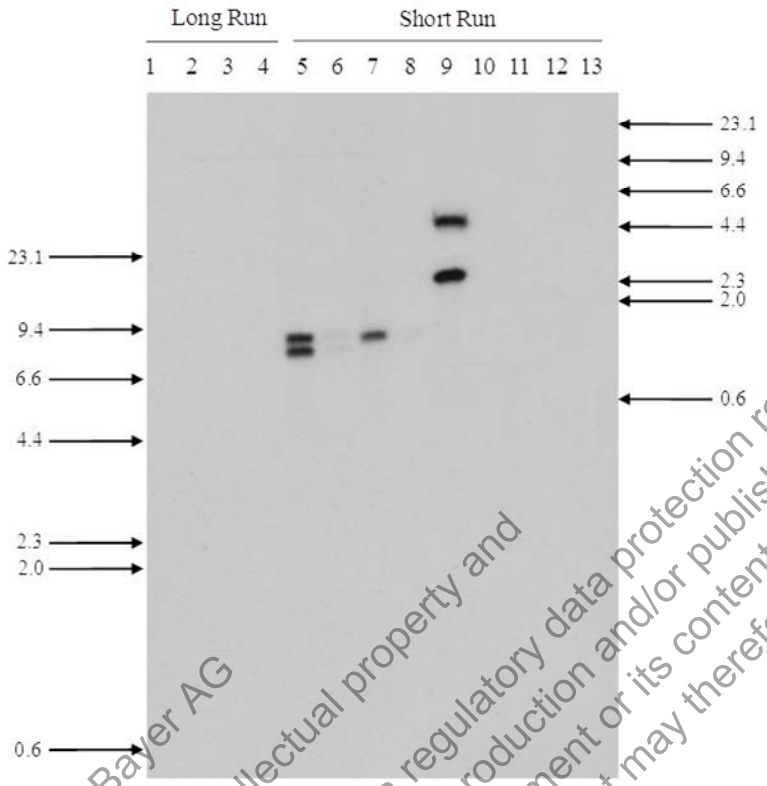


Figure IV-5. Southern Blot Analysis to Determine the Presence or Absence of PV-GHHT6997 Backbone Sequences in MON 88701: Probes 6, 7, and 8

The blot was hybridized with three ^{32}P-labeled probes that span the plasmid vector backbone sequences (Figure III-1, Probes 6, 7, and 8). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hind* III fragments on the ethidium bromide stained gel. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Bcl* I)
2	MON 88701 (*Bcl* I)
3	Conventional Control (*Ssp* I)
4	MON 88701 (*Ssp* I)
5	Conventional Control (*Bcl* I) spiked with Probe 7 and Probe 8 template [~1.0 genome
6	Conventional Control (*Bcl* I) spiked with Probe 7 and Probe 8 template [~0.1 genome
7	Conventional Control (*Bcl* I) spiked with Probe 6 template [~1.0 genome equivalent]
8	Conventional Control (*Bcl* I) spiked with Probe 6 template [~0.1 genome equivalent]
9	Conventional Control (*Bcl* I) spiked with PV-GHHT6997 (*Pci* I) [~1.0 genome equivalent]
10	Conventional Control (*Bcl* I)
11	MON 88701 (*Bcl* I)
12	Conventional Control (*Ssp* I)
13	MON 88701 (*Ssp* I)

IV.C. Organization and Sequence of the Insert and Adjacent Genomic DNA in MON 88701

The organization and sequence of the elements within the MON 88701 insert was confirmed by DNA sequence analysis. PCR primers were designed with the intent to amplify three overlapping DNA amplicons that span the entire length of the insert and the associated DNA flanking the 5′ and 3′ ends of the insert (Figure IV-6). The amplified PCR products were subjected to DNA sequence analyses. This analysis determined that the DNA sequence of the MON 88701 insert is 4105 bp long (Table IV-2) and is identical to the corresponding T-DNA sequence of PV-GHHT6997 as described in Table III-1.

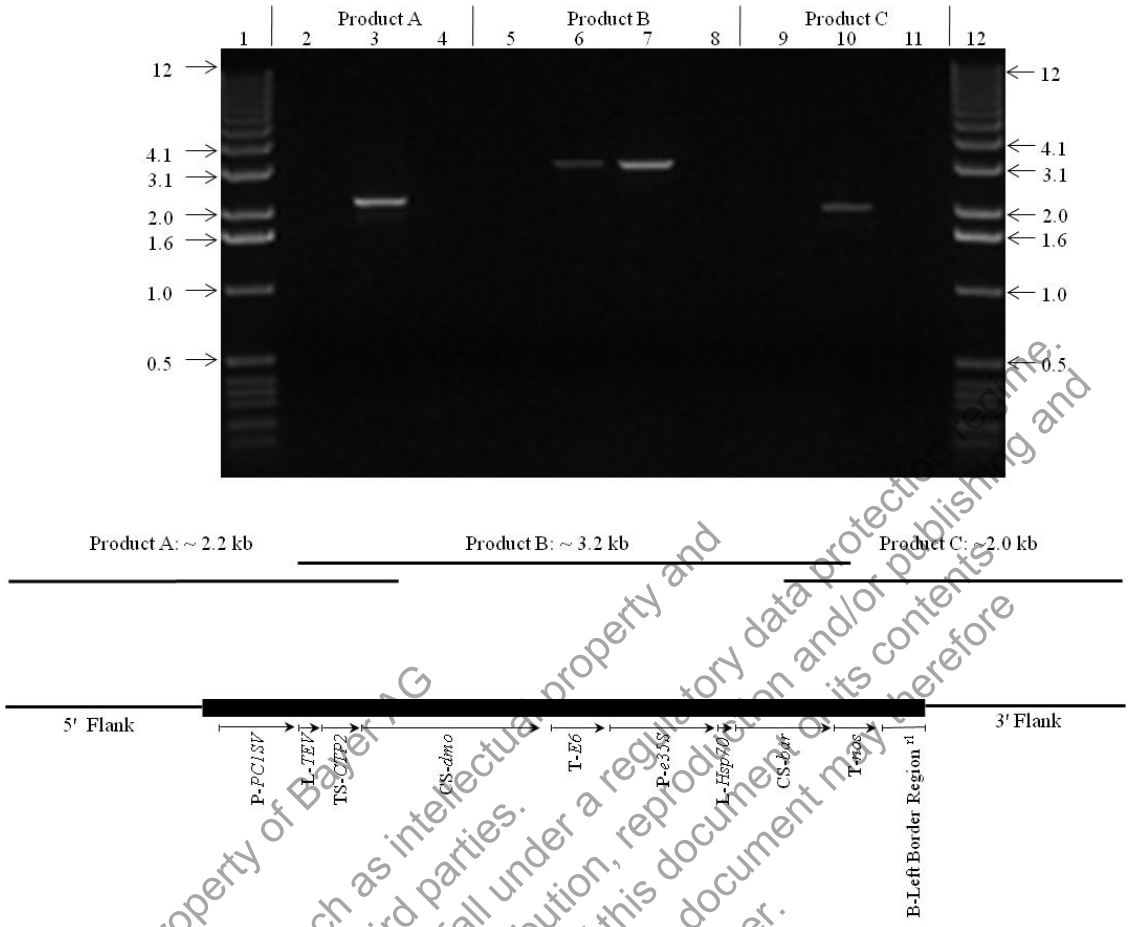


Figure IV-6. Overlapping PCR Analysis across the Insert in MON 88701

PCR was performed on both conventional control genomic DNA and MON 88701 genomic DNA using three pairs of primers to generate overlapping PCR fragments from MON 88701 for sequence analysis. Approximately five microliters of each of the PCR reactions was loaded on the gel. The expected product size for each amplicon and an illustration of the insert in MON 88701 is provided at the bottom of the figure. Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	Description
1	1 Kb DNA Ladder
2	Conventional Control
3	MON 88701
4	No template DNA control
5	Conventional Control
6	MON 88701
7	PV–GHHT6997
8	No template DNA control
9	Conventional Control
10	MON 88701
11	No template DNA control
12	1 Kb DNA Ladder

IV.D. PCR and DNA Sequence Analyses to Examine the MON 88701 Insertion Site

PCR and sequence analyses were performed on genomic DNA extracted from MON 88701 and the conventional control to examine the MON 88701 insertion site. The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5′ end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3′ end of the insert (Figure IV-7). The amplified PCR product from the conventional control was subjected to DNA sequence analysis. Alignments between the conventional control sequence obtained from this analysis and the sequences immediately flanking the 5′ and 3′ end of the MON 88701 insert were separately performed to determine the integrity and genomic organization of the insertion site in MON 88701. The alignment analyses indicated a 123 base pair deletion from the conventional genomic DNA occurred upon T-DNA insertion in MON 88701. Minor deletions and/or insertions of DNA due to double-strand break repair mechanisms in the plant during *Agrobacterium*-mediated transformation process are not uncommon (Salomon and Puchta, 1998).

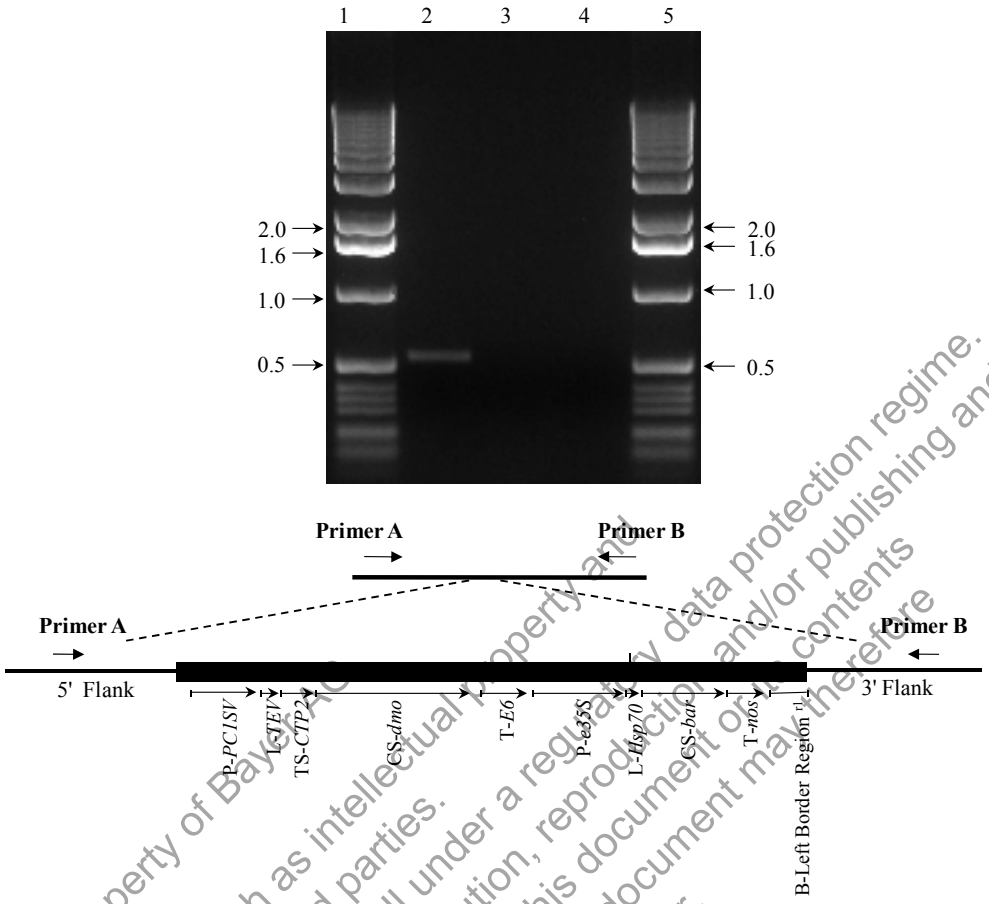


Figure IV-7. PCR Amplification of the MON 88701 Insertion Site in Conventional Control

PCR was performed on both conventional control genomic DNA and MON 88701 genomic DNA, using Primer A specific to the 5′ flanking sequence and Primer B specific to the 3′ flanking sequence of the insert in MON 88701, to generate DNA fragments for sequence analysis. The insertion site in the conventional control (top) and MON 88701 (bottom) are illustrated at the bottom of the figure. Approximately five microliters of each of the PCR reactions were loaded on the gel. Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	Description
1	1 Kb DNA Ladder
2	Conventional Control
3	MON 88701
4	No template DNA control
5	1 Kb DNA Ladder

IV.E. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 88701

In order to demonstrate the stability of the insert in MON 88701, Southern blot analysis was performed using genomic DNA extracted from leaf tissues from five breeding generations of MON 88701. For reference, the breeding history of MON 88701 is presented in Figure IV-8. The specific generations tested are indicated in the legend of Figure IV-8. The R_3 generation was used for the molecular characterization analyses shown in Figure IV-2 through Figure IV-5. To analyze insert stability, four samples from four additional generations of MON 88701 were evaluated by Southern blot analysis and compared to the R_3 generation. Genomic DNA, isolated from each of the selected generations of MON 88701, was digested with the restriction enzyme *Bcl* I and simultaneously hybridized with Probe 2 and Probe 4 (Figures III-1 and IV-1), which was designed to detect both fragments generated by the *Bcl* I digest. Any instability associated with the insert would be detected as extra bands within the fingerprint on the Southern blot. The Southern blot has the same controls as described in Section IV.A.2.

IV.E.1. T-DNA Probe 2 and 4

Conventional control genomic DNA digested with restriction enzyme *Bcl* I and simultaneously hybridized with Probe 2 and Probe 4 (Figures III-1 and IV-1) produced no hybridization signals (Figure IV-9, Lane 1) as expected for the negative control. Conventional control genomic DNA digested with *Bcl* I and spiked with the PV-GHHT6997 DNA, previously digested with the restriction enzyme *Pci* I (Figure III-1 and Table IV-1), produced one expected band at ~6.2 kb (Figure IV-9, Lane 2). Conventional control genomic DNA digested with *Bcl* I and spiked with probe templates of Probe 2 and Probe 4 produced the expected bands at ~1.0 kb and ~0.8 kb (Figure IV-9, Lane 3 and Lane 4). Detection of the positive controls indicates that the probes hybridized to their target sequences.

MON 88701 genomic DNA digested with *Bcl* I and hybridized with Probe 2 and Probe 4 (Figures III-1 and IV-1) is expected to produce a Southern fingerprint with two bands at ~3.5 kb and ~2.4 kb (Figure IV-1 and Table IV-1). Southern fingerprints produced from multiple generations (Figure IV-9, Lane 5, Lanes 7-9) of MON 88701 are consistent with the one produced from the fully characterized generation R_3 (Figure IV-3, Lane 2 and Lane 9, and Figure IV-9, Lane 6), indicating that MON 88701 contains one copy of the T-DNA insert that is stable across multiple generations.

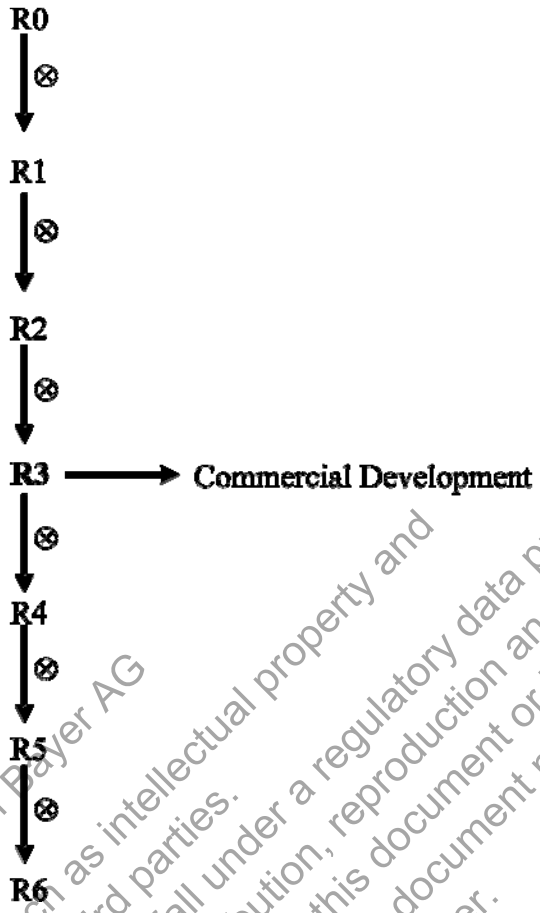


Figure IV-8. Breeding History of MON 88701

R_0 corresponds to the original transformed cotton plant. ⊗ designates self-pollination. The R_3 generation was used for the molecular characterization and commercial development of MON 88701. The R_2, R_3, R_4, R_5, and R_6 generations of MON 88701 were used to analyze the stability of the insert across generations. The R_5 generation was used for protein expression in tissues other than seed and for agronomic, phenotypic, and environmental interaction analyses. The R_6 generation was used for protein expression in seed and for composition analysis.

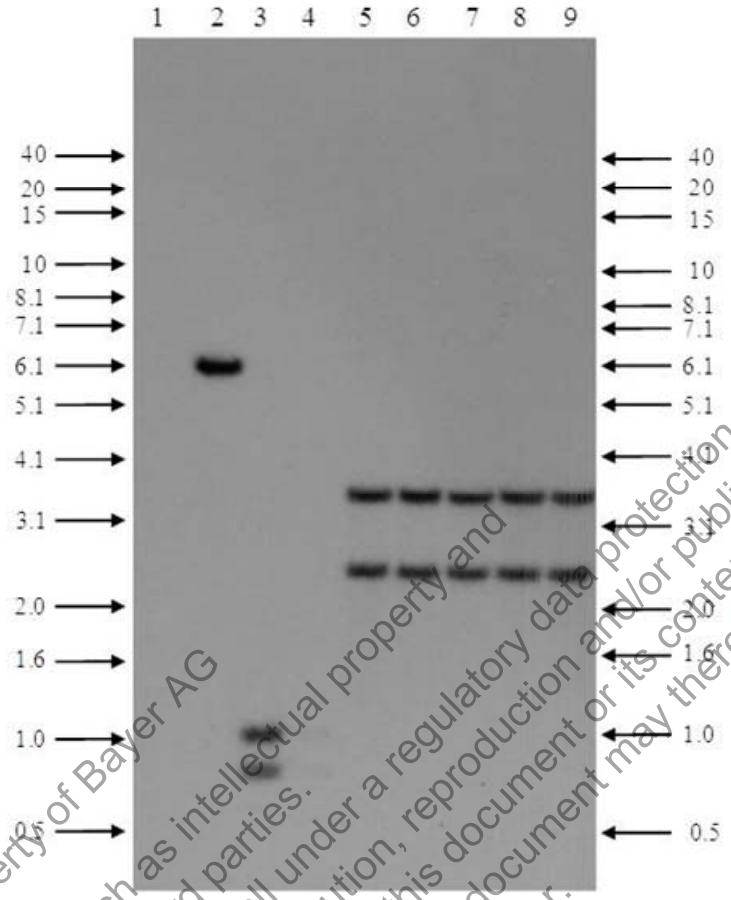


Figure IV-9. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 88701: Probes 2 and 4

The blot was simultaneously hybridized with two ^{32}P-labeled probes that span a portion of the T-DNA sequence (Figure III-1, Probe 2 and Probe 4). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from 1Kb DNA Extension Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane

1 Conventional control (*Bcl* I)
2 Conventional control (*Bcl* I) spiked with PV-GHHT6997 (*Pci* I) [~1.0 genome equivalent]
3 Conventional control (*Bcl* I) spiked with Probe 2 and Probe 4 template [~1.0 genome equivalent]
4 Conventional control (*Bcl* I) spiked with Probe 3 and Probe 4 template [~0.1 genome equivalent]
5 MON 88701 (R_2) (*Bcl* I)
6 MON 88701 (R_3) (*Bcl* I)
7 MON 88701 (R_4) (*Bcl* I)
8 MON 88701 (R_5) (*Bcl* I))
9 MON 88701 (R_6) (*Bcl* I)

IV.F. Inheritance of the Genetic Insert in MON 88701

The MON 88701 T-DNA resides at a single locus within the cotton genome and is inherited according to Mendelian principles of inheritance. During development of MON 88701, phenotypic and genotypic segregation data were recorded to assess the inheritance and stability of the MON 88701 T-DNA using Chi-square (χ^2) analysis over several generations. The χ^2 analysis is based on comparing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 88701 breeding path for generating pollinated segregation data is described in Figure IV-10. The transformed R_0 plant was self-pollinated to generate R_1 seed. The segregating R_1 generation was assessed using Real-Time TaqMan analysis for the *dmo* coding region. A single homozygous positive R_1 plant was selected and self-pollinated to give rise to R_2 plants that were self-pollinated to produce R_3 seed. Phenotypic and genotypic assays confirmed the lack of insert segregation in these self-pollinated generations.

Homozygous positive R_3 plants were crossed to a Monsanto proprietary cotton inbred, which does not contain the *dmo* or *bar* coding sequence, via traditional breeding techniques to produce hemizygous F_1 seed. The F_1 plants, hemizygous for the dicamba and glufosinate tolerant trait, were crossed with a Monsanto proprietary cotton inbred, which does not contain the *dmo* or *bar* coding sequence, to produce $BC1F_1$ seed. The $BC1F_1$ generation was assessed using a glufosinate herbicide application to select for plants containing the MON 88701 T-DNA. The plants that survived the herbicide application were confirmed to be hemizygous for the MON 88701 T-DNA using an event-specific End-Point TaqMan analysis. The hemizygous $BC1F_1$ plants were self-pollinated to produce the $BC1F_2$ plants. For the $BC1F_2$ generation, the plants were assessed using a glufosinate herbicide application and the surviving plants were assessed using an event-specific End-Point TaqMan analysis for the MON 88701 T-DNA.

The inheritance of the MON 88701 T-DNA was assessed in the R_1, $BC1F_1$, and $BC1F_2$ generations. At the $BC1F_1$ generation, the MON 88701 T-DNA was predicted to segregate at a 1:1 ratio (hemizygous: homozygous negative) according to Mendelian inheritance principles. At the R_1 and $BC1F_2$ generations, the MON 88701 T-DNA was predicted to segregate at a 1:2:1 ratio (homozygous positive: hemizygous: homozygous negative) according to Mendelian inheritance principles.

A Chi-square (χ^2) analysis was used to compare the observed segregation ratios of the MON 88701 T-DNA to the expected ratios. The Chi-square (χ^2) analysis used the statistical program R Version 2.12.0 (2010-10-15).

The Chi-square was calculated as:

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

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

The results of the χ^2 analysis of the MON 88701 segregating progeny are presented in Table IV-3 and Table IV-4. The χ^2 value in the $BC1F_1$ generation indicated no statistically significant difference between the observed and expected 1:1 segregation ratio (hemizygous: homozygous negative) of the MON 88701 T-DNA. The χ^2 value for the R_1 and $BC1F_2$ generations indicated no statistically significant difference between the observed and expected 1:2:1 segregation ratio (homozygous positive: hemizygous: homozygous negative) of MON 88701 T-DNA. These results support the conclusion that the MON 88701 T-DNA resides at a single locus within the cotton genome and is inherited according to Mendelian principles of inheritance. These results are also consistent with the molecular characterization data indicating that MON 88701 contains a single intact copy of the *dmo* and *bar* expression cassettes inserted at a single locus in the cotton genome.

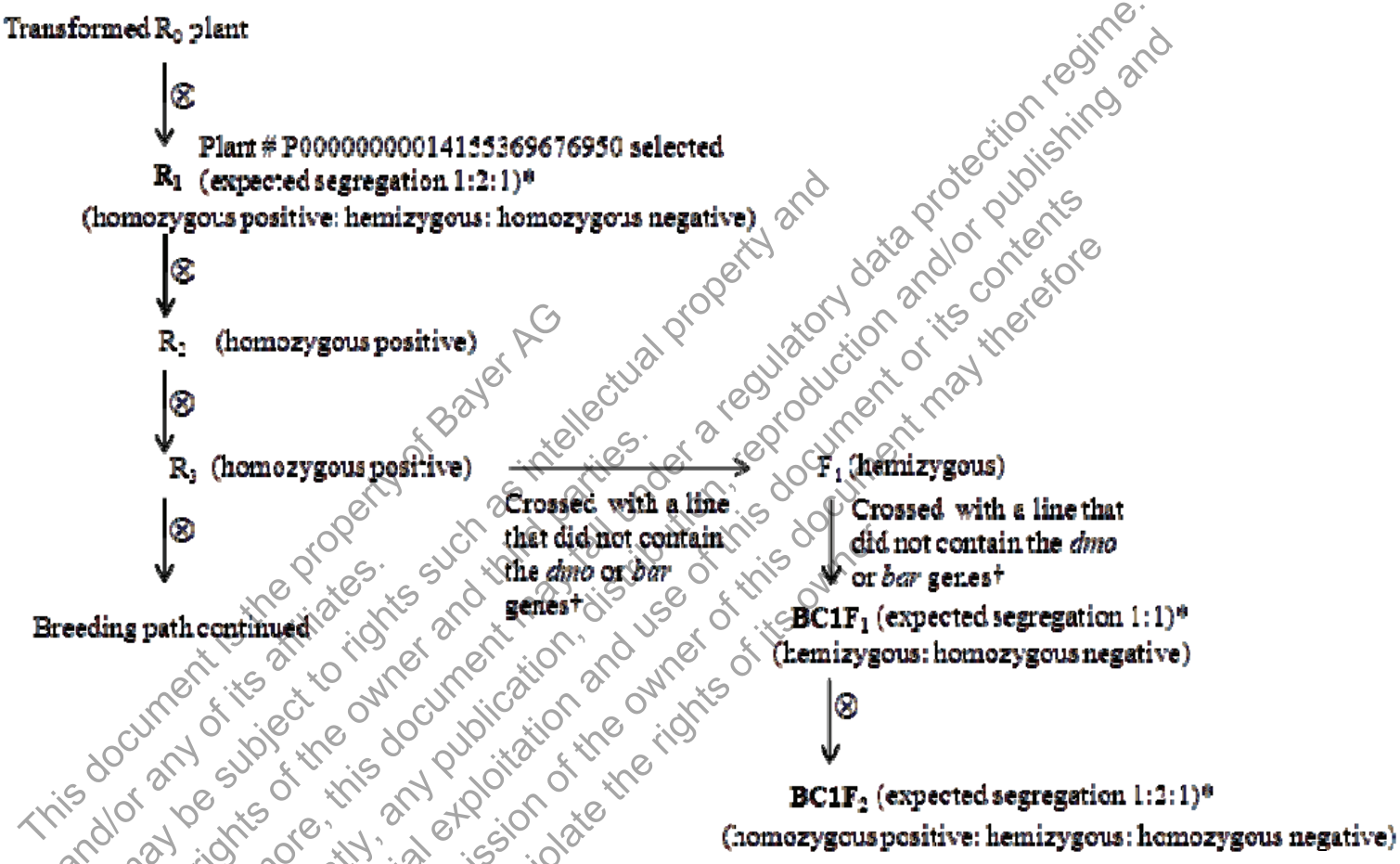


Figure IV-10. Breeding Path for Generating Segregation Data for MON 88701
*Chi-square analysis was conducted on segregation data from the R_1, $BC1F_1$, and $BC1F_2$ generations (bolded text).
†The cotton line used in the cross that did not contain the *dmo* or *bar* genes is a Monsanto proprietary cotton inbred.
⊗=Self- Pollinated

Table IV-3. Segregation of the T-DNA During the Development of MON 88701: 1:1 Segregation

				1:1 Segregation			
Generation	**Total Plants**	**Observed # Plants Hemizygous**	**Observed # Plants Homozygous Negative**	**Expected # Plants Hemizygous**	**Expected # Plants Homozygous Negative**	**χ^2**	**Probability[2]**
$BC1F_1$[1]	261	123	138	130.5	130.5	0.862	0.3532

[1] Segregation was evaluated using a glufosinate herbicide application followed by End-Point TaqMan analysis for the MON 88701 insert.
[2] Chi-square analysis was performed to analyze the segregation ratios ($p \leq 0.05$).

Table IV-4. Segregation of the T-DNA During the Development of MON 88701: 1:2:1 Segregation

					1:2:1 Segregation			
Generation	**Total Plants**	**Observed # Plants Homozygous Positive**	**Observed # Plants Hemizygous**	**Observed # Plants Homozygous Negative**	**Expected # Plants Homozygous Positive**	**Expected # Plants Hemizygous**	**Expected # Plants Homozygous Negative**	**χ^2**	**Probability[3]**
R_1[1]	173	33	99	41	43.25	86.50	43.25	4.353	0.1135
$BC1F_2$[2]	118	36	56	26	29.50	59.00	29.50	2.000	0.3679

[1] Segregation was evaluated using Real-Time TaqMan analysis for the *dmo* coding region.
[2] Segregation was evaluated using a glufosinate herbicide application followed by End-Point TaqMan analysis for the MON 88701 insert.
[3] Chi-square analysis was performed to analyze the segregation ratios ($p \leq 0.05$).

IV.G. Genetic Modification Characterization Conclusion

Molecular characterization of MON 88701 by Southern blot analyses confirmed that the T-DNA was inserted into the cotton genome at a single locus containing one copy of the *dmo* and *bar* expression cassettes. No backbone DNA sequences from PV-GHHT6997 were detected in MON 88701.

PCR and DNA sequence analyses performed on MON 88701 and the conventional control determined the following: the complete DNA sequence of the insert and the DNA sequences flanking the 5′ and 3′ ends of the insert in MON 88701; the organization of the genetic elements within the insert; and the 5′ and 3′ insert-to-genomic DNA junctions. The PCR and DNA sequence analysis also determined the DNA sequence at the insertion site in the conventional control and identified a rearrangement (123 base pair deletion) that occurred at the insertion site in MON 88701. Minor deletions and/or insertions of DNA due to double-strand break repair mechanisms in the plant during *Agrobacterium*-mediated transformation process are not uncommon (Salomon and Puchta, 1998).

Southern blot analysis of multiple MON 88701 generations demonstrated that the inserted DNA has been stably maintained through five generations of breeding, thereby, confirming the stability of the insert. Results from segregation analyses show inheritance and stability of the insert was as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 88701 at a single chromosomal locus.

V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE MON 88701 DMO AND PAT (*bar*) PROTEINS PRODUCED IN MON 88701

Characterization of the introduced protein(s) in a biotechnology-derived crop is important to establishing food, feed, and environmental safety. As described in Section IV, MON 88701 contains *dmo* and *bar* expression cassettes that, when transcribed and translated, result in the expression of the MON 88701 DMO and PAT (*bar*) proteins, respectively. This section summarizes: 1) the identity and function of the MON 88701 DMO and PAT (*bar*) proteins produced in MON 88701; 2) the demonstration of equivalence between the plant-produced and *E. coli*-produced proteins, which were used in various protein safety studies; 3) the expression levels of the MON 88701 DMO and PAT (*bar*) proteins in MON 88701 plant tissues; 4) the assessment of the potential allergenicity of the MON 88701 DMO and PAT (*bar*) proteins produced in MON 88701; and 5) the food, feed, and environmental safety assessment of the MON 88701 DMO and PAT (*bar*) proteins produced in MON 88701. The data support a conclusion that these two proteins produced in MON 88701 are safe for the environment and human or animal consumption based on several lines of evidence summarized below. These data were supplied to FDA for their evaluation in consultation BNF No. 135 on the food and feed safety and compositional assessment of MON 88701.

V.A. Identity and Function of the MON 88701 DMO and PAT (*bar*) Proteins from MON 88701

V.A.1. Mode-of-Action of DMO and MON 88701 DMO

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

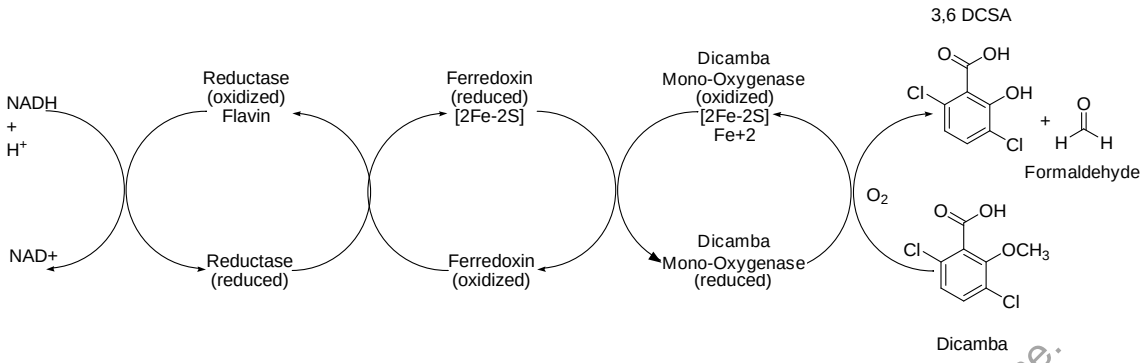


Figure V-1. Three Components of the DMO Oxygenase System

The crystal structure of a DMO has been solved (D'Ordine et al., 2009; Dumitru et al., 2009) and shows that the DMO monomers contain a Rieske [2Fe-2S] cluster domain and a non-heme iron center domain typical of all Rieske-type mono-oxygenases (Ferraro et al., 2005). To catalyze the demethylation of dicamba, electrons transferred from NADH are shuttled through an endogenous reductase and ferredoxin to the terminal DMO. The electrons are received by the Rieske [2Fe-2S] cluster on one DMO monomer and transferred to the non-heme iron center at the catalytic site of an adjacent monomer (D'Ordine et al., 2009; Dumitru et al., 2009), where it reductively activates oxygen to catalyze the final demethylation of dicamba. As a result of the reaction, 3,6-dichlorosalicylic acid (DCSA) and formaldehyde are formed. DCSA is a known cotton, soybean, soil, and livestock metabolite whose safety has been evaluated by the EPA (FAO-WHO, 2011a; 2011b; U.S. EPA, 2009). Formaldehyde is found naturally in many plants at levels up to several hundred ppm (Adrian-Romero et al., 1999). An assessment of the safety and potential effects of the DMO reaction products is provided in Appendix C.1.

V.A.1.1. Description of MON 88701 DMO

DMO is targeted to chloroplasts for co-localization with the endogenous reductase and ferredoxin enzymes that supply electrons for the DMO demethylation reaction as described by Behrens et al. (2007). In the construction of the plasmid vector used in the development of MON 88701, PV-GHHT6997, a transit peptide coding sequence (*CTP2,* Table IV-2) was joined to the *dmo* coding sequence; this coding sequence results in the production of a precursor protein consisting of the DMO protein and an additional 76 amino acids at the N-terminus of the protein. These additional amino acids correspond to the chloroplast transit peptide (CTP) from *Arabidopsis thaliana* EPSPS (*CTP2*), which is incorporated to improve the targeting of the precursor protein to the chloroplast (Herrmann, 1995; Klee et al., 1987). Typically, transit peptides are precisely removed from the precursor protein following delivery to the targeted plastid (Della-Cioppa et al., 1986) resulting in the full-length protein. However, there are examples in the literature of alternatively processed forms of a protein targeted to a plant's chloroplast (Behrens et al., 2007; Clark and Lamppa, 1992). Such alternative processing is observed with the MON 88701 DMO protein produced in MON 88701.

Analysis of cottonseed extracts from MON 88701 determined that the expressed protein had an apparent molecular weight of 39.5 kDa and corresponded to the DMO protein with nine amino acids on the N-terminus originating from the EPSPS chloroplast transit peptide. Except for the 9 amino acids derived from the CTP2 and an additional leucine at position two, the MON 88701 DMO protein has an identical sequence to the wild-type DMO protein from the DI-6 strain of *S. maltophilia* (Herman et al., 2005). The differences in the amino acid sequence between the wild-type DMO protein and MON 88701 DMO protein are not expected to have an effect on structure, activity, or specificity because the N-terminus and position two are sterically distant from the catalytic site (D'Ordine et al., 2009; Dumitru et al., 2009). The DMO protein produced in MON 88701 is hereinafter referred to as MON 88701 DMO protein. Accordingly, the DMO protein produced from *E. coli* with the same sequence as MON 88701 DMO is referred to as *E. coli*-produced MON 88701 DMO protein.

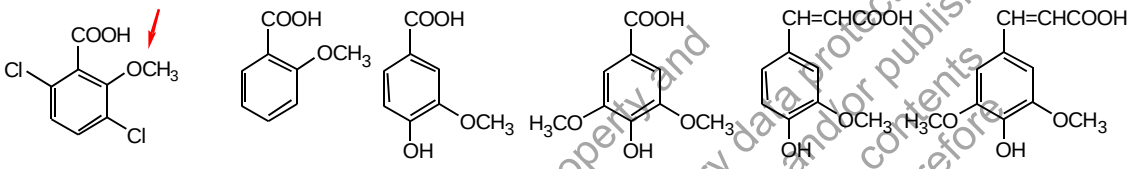
As described previously the active form of DMO is a trimer (Chakraborty et al., 2005; Dumitru et al., 2009). For MON 88701 DMO to be functionally active and confer dicamba tolerance to MON 88701, a trimeric structure is required. The activity of MON 88701 DMO was confirmed during characterization (Section V.B and Appendix C).

V.A.1.2. Specificity of MON 88701 DMO

The substrate specificity of MON 88701 DMO was evaluated to understand potential interactions DMO may have with potential substrates present in MON 88701 cotton. The literature indicates the specificity of DMO for dicamba is due to the specific interactions that occur at the catalytic site (D'Ordine et al., 2009; Dumitru et al., 2009). Dicamba interacts with amino acids in the catalytic site of DMO through both the carboxylate moiety and the chlorine atoms of dicamba, which are primarily involved in orienting the substrate in the catalytic site. These chlorine atoms are required for catalysis (D'Ordine et al., 2009; Dumitru et al., 2009). Given the limited existence of chlorinated compounds with structures similar to dicamba in plants and other eukaryotes (Wishart, 2010; Wishart et al., 2009), it is unlikely that MON 88701 DMO will catalyze the conversion of other endogenous substrates.

The potential for MON 88701 DMO to metabolize endogenous plant substrates was evaluated through *in vitro* experiments using a purified N-terminal histidine tagged DMO that was identical to wild-type DMO, except for a histidine tag at the N-terminus added to aid in protein purification. A comparison of DMO versions is shown in Appendix C, Figure C-1. A set of potential endogenous substrates was selected for evaluation based on structural similarity of the compounds to dicamba and their presence in cotton, corn, and soybean (Buchanan et al., 2000; Janas et al., 2000; Lege et al., 1995; Schmelz et al., 2003). The potential substrates tested were *o*-anisic acid (2-methoxybenzoic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid), syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid), ferulic acid [3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid] and sinapic acid [3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid] (Figure V-2). The assay mixture included NADH, reductase, ferredoxin and DMO. Dicamba was first used as a positive control to demonstrate that the assay system was

functional. The disappearance of potential substrates and the formation of potential oxidation products were monitored using LC-UV and LC-MS (Appendix C). None of the tested substrates, except dicamba, were metabolized by the histidine tagged DMO in these *in vitro* experiments. To assess whether MON 88701 DMO protein has the same specificity as the histidine tagged DMO used in the *in vitro* experiments, the *E. coli*-produced MON 88701 DMO protein (*i.e.*, lacking a histidine), shown to be equivalent to the plant produced MON 88701 DMO protein (Section V.B), was incubated with *o*-anisic acid, the endogenous compound that has the greatest structural similarity to dicamba. Again dicamba was used as a positive control to demonstrate the assay system was functional. This analysis demonstrated that *o*-anisic acid was not metabolized by the *E. coli*-produced MON 88701 DMO protein (*i.e.*, lacking a histidine), but dicamba was. These results indicate that DMO, including the MON 88701 DMO protein, is specific for dicamba as a substrate (See Section V.E.1.3 and Appendix C.3.2 for additional details).



Dicamba o-Anisic Acid Vanillic Acid Syringic Acid Ferulic Acid Sinapic Acid

Figure V-2. Dicamba and Potential Endogenous Substrates Tested through *In Vitro* Experiments with DMO
The arrow indicates methyl group removed by DMO.

The possibility that MON 88701 DMO can metabolize exogenous substrates was tested through *in vivo* greenhouse experiments. In addition to dicamba, nine other herbicides, representing eight families with distinct modes-of-action, some of which are approved for use in cotton, were tested with MON 88701 and the conventional control (Table V-1). Each herbicide was applied at two spray rates that are representative of potential commercial rates needed to control broadleaf weeds. Herbicides were applied preemergence or at the 2 to 5 leaf plant growth stage and plants were scored with a visual rating based on the amount of injury observed. Across all of the herbicides tested, MON 88701 and the conventional control were similar in their level of injury, indicating that these herbicides do not serve as a substrate for MON 88701 DMO (Appendix C.3.).

Table V-1. Herbicides Applied to MON 88701 and Conventional Control

Herbicide Active Ingredient	Herbicide Chemical Family (Mode-of-Action)[1]
Dicamba	Benzoic (Synthetic Auxin)
2,4-D	Phenoxycarboxylic acid (Synthetic Auxin)
2,4-DB	Phenoxycarboxylic acid (Synthetic Auxin)
Acetochlor	Chloroacetamide (Inhibition of VLCFAs)
Atrazine	Triazine (Inhibition of Photosynthesis at Photosytem II)
Oxyfluorfen	Diphenylether (Inhibition of PPO)
Halosulfuron	Sulfonylurea (Inhibition of ALS)
Trifluralin	Dinitroaniline (Microtubule Assembly Inhibition)
Paraquat	Bipyridilium (Photosystem I electron diversion)
Glyphosate	Glycine (Inhibition of EPSP synthase)

[1] (HRAC, 2009)
[2] 2,4-D = 2,4-Dichlorophenoxyacetic acid; 2,4-DB = 4-(2,4-dichlorophenoxy) butyric acid.

V.A.2. Mode-of-Action of PAT Proteins

The mode-of-action for PAT protein has been extensively assessed, as numerous glufosinate-tolerant products including those in cotton, corn, soy, canola, sugarbeet and rice have been reviewed by the FDA and several other regulatory agencies (ILSI-CERA, 2011; OECD, 1999a; 2002a). PAT, including the PAT (*bar*) protein produced in MON 88701, is an enzyme classified as an acetyltransferase which acetylates glufosinate to produce non-herbicidal N-acetyl glufosinate. Glufosinate is a racemic mixture of the D- and L- forms of phosphinothricin, though only the L-form has herbicidal activity. The herbicidal activity of glufosinate results from the binding of L-phosphinothricin to glutamine synthetase (OECD, 1999b; 2002a). Glutamine synthetase is responsible for the assimilation of ammonia generated during photorespiration. The binding of L-phosphinothricin to glutamine synthetase results in the inactivation of glutamine synthetase and a subsequent toxic build-up of ammonia within the plant, resulting in death of the plant (Manderscheid and Wild, 1986; OECD, 1999b; 2002a; Wild and Manderscheid, 1984).

The PAT (*bar*) protein produced in MON 88701 acetylates the free amine group of L-phosphinothricin form of glufosinate to produce non-herbicidal N-acetyl glufosinate. The acetylated glufosinate is unable to bind to glutamine synthetase and therefore does not disrupt photorespiration and avoids the build-up of ammonia. Therefore, the production of PAT (*bar*) protein in MON 88701 confers glufosinate herbicide tolerance through this mechanism.

V.A.2.1. Description of PAT (*bar*)

Phosphinothricin N-acetyltransferase (PAT) proteins conferring tolerance to glufosinate herbicide (2-amino-4-(hydroxymethylphosphinyl) butanoic acid) have been isolated from two separate species of *Streptomyces*, *S. hygroscopicus* ([redacted] et al., 1987) and *S. viridochromogenes* (Wohlleben et al., 1988). The PAT protein isolated from

S. hygroscopicus is encoded by the *bar* gene, and the PAT protein isolated from *S. viridochromogenes* is encoded by the *pat* gene. These PAT proteins are made up of 183 amino acids with 85% identity at the amino acid level. Based on previous studies (Wehrmann et al., 1996) that have extensively characterized PAT proteins produced from *bar* and *pat* genes, OECD recognizes both proteins to be equivalent with regard to function and safety (OECD, 1999b). In addition, EPA has issued a tolerance exemption for PAT protein regardless of the encoding gene (U.S. EPA, 1997). The safety of PAT proteins present in biotechnology-derived crops has been extensively assessed (Hérouet et al., 2005; ILSI-CERA, 2011).

The PAT protein produced in MON 88701 is from the *bar* gene, and for clarity, the PAT protein produced in MON 88701 will be referred to as PAT (*bar*). Analysis of cottonseed extracts from MON 88701 determined that the expressed protein corresponded to the 183 amino acid polypeptide, resulting in a 24.1 kDa PAT (*bar*) protein. The activity of the PAT (*bar*) protein purified from MON 88701 cottonseed was confirmed during characterization (Appendix C.4.).

V.A.2.2. PAT (*bar*) Specificity

The PAT proteins, including PAT (*bar*), are highly specific for glufosinate in the presence of acetyl-CoA ([redacted] et al., 1987; Wehrmann et al., 1996). While the herbicidal activity of glufosinate comes from the L-amino acid form, other L-amino acids are unable to be acetylated by PAT protein and competition assays containing glufosinate, high concentrations of other amino acids and PAT showed no inhibition of glufosinate acetylation (Wehrmann et al., 1996). Furthermore, L-glutamate, an analogue of glufosinate, also showed no inhibition of glufosinate acetylation in competition assays (Wehrmann et al., 1996). In addition, the PAT (*bar*) protein has more than 30-fold higher affinity towards L-phosphinothricin over other analogues ([redacted] et al., 1987). Thus, the PAT (*bar*) protein has high substrate specificity for L-phosphinothricin, the herbicidal component of glufosinate, and it is unlikely to affect the metabolic system of MON 88701 cotton. Numerous glufosinate-tolerant products including those in cotton, corn, soy, canola, sugarbeet, and rice have been reviewed with no concerns identified (ILSI-CERA, 2011).

V.B. Characterization and Equivalence of MON 88701 DMO and PAT (*bar*) Proteins from MON 88701

The safety assessment of crops derived through biotechnology includes characterization of the physicochemical and functional properties of the protein(s) produced from the inserted DNA, and confirmation of the safety of the protein(s). For the safety data generated using *E. coli*-produced protein(s) to be applied to plant-produced protein(s), the equivalence of the plant- and *E. coli*-produced proteins must be assessed. For MON 88701 the physicochemical and functional characteristics of the MON 88701 DMO and MON 88701-produced PAT (*bar*) proteins were determined and each was shown to be equivalent to its respective *E. coli*-produced protein. A summary of the analytical results for each protein are shown below and the details of the materials, methods, and results are described in Appendix C.

The MON 88701 DMO protein purified from cottonseed of MON 88701 was characterized and the equivalence of the physicochemical and functional properties between the MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins was established using a panel of analytical tests: 1) the identity could not be confirmed by N-terminal sequence analysis; however, MALDI-TOF MS analysis of peptides derived from tryptic digested MON 88701 DMO established the N-terminal sequence of MON 88701 DMO; 2) MALDI-TOF MS analysis yielded peptide masses consistent with the expected peptide masses from the theoretical trypsin digest of the MON 88701 DMO sequence; 3) MON 88701 DMO protein was detected on a western blot probed with antibodies specific for DMO protein and the immunoreactive and physicochemical properties of the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were shown to be equivalent; 4) the electrophoretic mobility and apparent molecular weight of the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were shown to be equivalent; 5) glycosylation status of MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were determined to be equivalent; and 6) functional activity of the MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins were demonstrated to be equivalent.

The MON 88701-produced PAT (*bar*) protein purified from cottonseed of MON 88701 was characterized and the equivalence of the immunoreactive and physicochemical characteristics and functional activity between the MON 88701- and the *E. coli*-produced PAT (*bar*) proteins was established using a panel of analytical tests: 1) N-terminal sequence analysis of the MON 88701-produced PAT (*bar*) protein established identity; 2) MALDI-TOF MS analysis yielded peptide masses consistent with the expected peptide masses from the theoretical trypsin digest of the MON 88701-produced PAT (*bar*) sequence; 3) MON 88701-produced PAT (*bar*) protein was detected on a western blot probed with antibodies specific for PAT (*bar*) protein and the immunoreactive properties of the MON 88701-produced and *E. coli*-produced PAT (*bar*) proteins were shown to be equivalent; 4) the electrophoretic mobility and apparent molecular weight of the MON 88701-produced and *E. coli*-produced PAT (*bar*) proteins were shown to be equivalent; 5) glycosylation status of MON 88701- and *E. coli*-produced MON 88701 PAT (*bar*) proteins were determined to be equivalent; and 6) functional activity of the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were demonstrated to be equivalent.

Taken together, these data provide a detailed characterization of the MON 88701 DMO and PAT (*bar*) proteins and establish their respective equivalence to *E. coli*-produced MON 88701 DMO protein and *E. coli*-produced PAT (*bar*) protein. This equivalence justifies the use of the *E. coli*-produced proteins as test subtances in the protein safety studies.

V.C. Expression Levels of MON 88701 DMO and PAT (*bar*) Proteins in MON 88701

MON 88701 DMO and PAT (*bar*) protein levels in various tissues of MON 88701 relevant to the risk assessment were determined by a validated enzyme-linked immunosorbent assay (ELISA). Tissues of MON 88701 were collected from four

replicate plots planted in a randomized complete block field design during the 2010 growing season from the following eight field sites in the U.S.: Arkansas (ARTI), Georgia (GACH), Kansas (KSLA), Louisiana (LACH), North Carolina (NCBD), New Mexico (NMLC), South Carolina (SCEK), and Texas (TXPL). MON 88701 plots were treated at the 3-5 leaf stage with glufosinate herbicide at the label rate (0.5 lbs active ingredient [a.i.]/acre) and at the 6-10 leaf stage with dicamba herbicide at the proposed label rate (0.5 lbs acid equivalent [a.e.]/acre). The field sites were representative of cotton-producing regions suitable for commercial production. Seed, pollen, root, and overseason leaf (OSL-1 through OSL-4) tissue samples were collected from each replicated plot at all field sites.

V.C.1. Expression Levels of MON 88701 DMO Protein

MON 88701 DMO protein levels were determined in all seven tissue types. The results obtained from ELISA are summarized in Table V-2 and the details of the materials and methods are described in Appendix D. Due to a limited amount of tissue, moisture content was not measured for pollen; therefore, pollen is reported on a fresh weight (fw) basis only. MON 88701 DMO protein levels in MON 88701 across tissue types ranged from <LOD to 410 µg/g dw. The mean MON 88701 DMO protein levels were determined across eight sites, with the exception of OSL-1 (7 sites) and OSL-4 (7 sites). Samples <LOD were not included in mean determinations. The mean MON 88701 DMO protein levels were highest in leaf (ranging from OSL-2 and OSL-3 at 240 µg/g dw, OSL-4 at 230 µg/g dw to OSL-1 at 180 µg/g dw), followed by root at 43 µg/g dw, seed at 21 µg/g dw, and pollen at 14 µg/g fw.

Table V-2. Summary of MON 88701 DMO Protein Levels in Tissues from MON 88701 Grown in 2010 U.S. Field Trials

Tissue[1]	Development Stage[2]	Days After Planting (DAP)	MON 88701 DMO Mean (SD) Range (µg/g fw)[3]	MON 88701 DMO Mean (SD) Range (µg/g dw)[4]	LOQ/LOD[5] (µg/g fw)
OSL-1	2-4 leaf	14-25	27 (7.6) 13 – 42	180 (52) 110 – 280	0.168/0.313
OSL-2	4-7 leaf	25-37	41 (12) 19 – 65	240 (69) 110 – 380	0.168/0.313
OSL-3	9 leaf - Full flower	35-99	52 (17) 24 – 97	240 (75) 91 – 410	0.168/0.313
OSL-4	Cutout – Full flower	70-121	57 (18) 0.70 – 91	230 (59) 2.8 – 310	0.168/0.313
Root	50% open flower – Full flower	62-99	14 (3.7) 8.2 – 21	43 (12) 26 – 72	0.136/0.313
Pollen	50% open flower – Full Flower	68-99	14 (28) 0.31 – 110	NA (NA) NA	0.043/0.125
Seed	Maturity	148-183	20 (4.6) 8.2 – 29	21 (5.0) 8.9 – 33	0.059/0.313

[1]OSL= overseason leaf. Seed = black seed (ginned and delinted).
[2]The crop development stage each tissue was collected ([redacted] et al., 2007).
[3]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fw). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=32, except OSL-3 n=31 due to one sample <LOD, OSL-1 and OSL-4 n=28 due to missed sample collections, and pollen n=29 due to two samples expressing <LOD and one being inconclusive).
[4]Protein levels are expressed as µg/g on a dry weight (dw) basis. The dry weight values were calculated by dividing the µg/g fw by the dry weight conversion factors obtained from moisture analysis data. NA= Not Applicable.
[5]LOQ=limit of quantitation; LOD=limit of detection.

V.C.2. Expression Levels of PAT (*bar*) Protein

PAT (*bar*) protein levels were determined in all seven tissue types. The results obtained from ELISA are summarized in Table V-3 and the details of the materials and methods are described in Appendix D. Due to a limited amount of tissue, moisture content was not measured for pollen; therefore, pollen is reported on a fresh weight (fw) basis only. PAT (*bar*) protein levels in MON 88701 across tissue types ranged from <LOQ to 10 µg/g dw. The mean PAT (*bar*) protein levels were determined across eight sites, with the exception of OSL-1 (7 sites) and OSL-4 (7 sites). Samples <LOD were not included in mean determinations. The mean PAT (*bar*) protein levels were highest in seed at 6.6 µg/g dw, followed by leaf (ranging from OSL-2 at 6.4 µg/g dw to OSL-4 at 3.2 µg/g dw), root at 1.8 µg/g dw, and pollen at 0.56 µg/g fw.

Table V-3. Summary of PAT (*bar*) Protein Levels in Tissues from MON 88701 Grown in 2010 U.S. Field Trials

Tissue[1]	Development Stage[2]	Days After Planting (DAP)	PAT (*bar*) Mean (SD) Range (µg/g fw)[3]	PAT (*bar*) Mean (SD) Range (µg/g dw)[4]	LOD/LOQ[5] (µg/g fw)
OSL-1	2-4 leaf	14-25	0.84 (0.21) 0.46 – 1.4	5.5 (1.5) 3.7 – 9.1	0.162/0.188
OSL-2	4-7 leaf	25-37	1.1 (0.26) 0.68 – 1.6	6.4 (1.4) 3.8 – 9.4	0.162/0.188
OSL-3	9 leaf – Full flower	35-99	1.0 (0.34) 0.34 – 1.7	4.8 (2.0) 1.3 – 10	0.162/0.188
OSL-4	Cutout – Full flower	70-121	0.78 (0.29) 0.42 – 1.7	3.2 (1.2) 2.0 – 6.7	0.162/0.188
Root	50% open flower-Full flower	62-99	0.56 (0.18) 0.27 – 0.89	1.8 (0.75) 0.93 – 3.3	0.096/0.188
Pollen	50% open flower – Full flower	68-99	0.56 (0.24) 0.27 – 0.90	NA (NA) NA	0.021/0.188
Seed	Maturity	148-183	6.1 (0.95) 4.8 – 8.8	6.6 (1.1) 5.2 – 9.6	0.032/0.188

[1]OSL= overseason leaf. Seed = black seed (ginned and delinted).

[2]The crop development stage each tissue was collected ([redacted] et al., 2007).

[3]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fw). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=32, except OSL-1 n=28 due to missed sample collections, OSL-4 n=27 due to missed sample collections and one sample expressing <LOD, OSL-3 n=31 due to one sample expressing <LOD, and pollen n=6 due to 26 samples expressing <LOQ).

[4]Protein levels are expressed as µg/g on a dry weight (dw) basis. The dry weight values were calculated by dividing the µg/g fw by the dry weight conversion factors obtained from moisture analysis data. NA= Not Applicable.

[5]LOQ=limit of quantitation; LOD=limit of detection.

V.D. Assessment of Potential Allergenicity of the MON 88701 DMO and PAT (*bar*) Proteins

Assessing the potential allergenicity of the expressed proteins is less relevant to MON 88701 since only cottonseed oil and linters from cotton are used in food applications, which have undetectable or negligible amounts of total protein ([redacted] and [redacted], 1979; Sims et al., 1996). Nonetheless, the allergenic potential of MON 88701 DMO and PAT (*bar*) proteins was assessed by comparing the biochemical characteristics of these introduced proteins to biochemical characteristics of known allergens (Codex Alimentarius, 2009). A protein is not likely to be associated with allergenicity if: 1) the protein is from a non-allergenic source; 2) the protein represents a very small portion of the total plant protein; 3) the protein does not share structural similarities to known allergens based on the amino acid sequence; and 4) the protein is rapidly digested in mammalian gastrointestinal systems.

V.D.1. Assessment of Potential Allergenicity of the MON 88701 DMO Protein

MON 88701 DMO has been assessed for its potential allergenicity according to the safety assessment guidelines described above, and conclusions were as follows:

1) MON 88701 DMO originates from *S. maltophilia*, an organism that has not been reported to be a source of known allergens.

2) MON 88701 DMO represents no more than 0. 008% of the total protein in the cottonseed of MON 88701[4]. Therefore, the MON 88701 DMO protein represents a very small portion of the total protein in the cottonseed of MON 88701 and due to the harsh conditions used in cottonseed processing is most likely absent in the oil and linters that are used for food production.

3) Bioinformatics analyses demonstrated that the MON 88701 DMO does not share amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes.

4) *In vitro* digestive fate experiments conducted with the MON 88701 DMO demonstrate that the proteins are rapidly digested in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF).

Taken together, these data support the conclusion that MON 88701 DMO does not pose a significant allergenic risk.

V.D.2. Assessment of Potential Allergenicity of the PAT (*bar*) Protein

The non-allergenic nature of PAT (*bar*) protein is established in the scientific literature (Hérouet et al., 2005) and by the tolerance exemption set by U.S. EPA (1997).

[4] % protein = (Mean level of protein expression (µg/g)/ Mean dry weight of total protein in seed µg/g) x 100 %

Furthermore, the safety of PAT proteins, including the PAT (*bar*) protein produced in MON 88701, has been assessed extensively by regulatory agencies in 11 different countries for more than 38 biotechnology-derived events in eight different species (ILSI-CERA, 2011). In addition, potential allergenicity of PAT (*bar*) protein produced in MON 88701 has been assessed according to the safety assessment guidelines described above, and conclusions were as follows.

1) PAT (*bar*) originates from *S. hygroscopicus*, an organism that has not been reported to be a source of known allergens.

2) PAT (*bar*) represents no more than 0. 002% of the total protein in the cottonseed of MON 88701.[5] Therefore, the PAT (*bar*) protein represents a very small portion of the total protein in the cottonseed of MON 88701 and due to the harsh conditions used in cottonseed processing is most likely absent in the oil and linters that are used for food production.

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

4) *In vitro* digestive fate experiments conducted with the PAT (*bar*) demonstrate that the proteins are rapidly digested in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF).

Taken together, these data support the conclusion that PAT (*bar*) does not pose a significant allergenic risk.

V.E. Safety Assessment Summary of MON 88701 DMO and PAT (*bar*) Proteins in MON 88701

Characterization of the introduced protein(s) in a biotechnology-derived crop product is important to establishing its food, feed, and environmental safety. This section summarizes: 1) the functionality of MON 88701 DMO and PAT (*bar*); 2) the characterization of MON 88701 DMO and PAT (*bar*); 3) the levels of MON 88701 DMO and PAT (*bar*) in plant tissues; 4) assessment of the potential allergenicity of MON 88701 DMO and PAT (*bar*); and 5) the food, feed, and environmental safety assessment of MON 88701 DMO and PAT (*bar*). The data support a conclusion that MON 88701 is safe for the environment and human or animal consumption based on several lines of evidence, all of which are summarized below.

V.E.1. MON 88701 DMO Donor Organism, History of Safe Use, and Specificity

Numerous factors have been considered in the safety assessment of MON 88701 DMO, which include but are not limited to donor organism safety, the safety of mono-

[5] % protein = (Mean level of protein expression (µg/g)/ Mean dry weight of total protein in seed µg/g) x 100 %

oxygenases, and MON 88701 DMO protein specificity. A comprehensive food, feed, and environmental safety assessment of the MON 88701 DMO was conducted. The results are summarized below, along with the conclusions reached from the assessment.

V.E.1.1. The *dmo* Donor Organism is Safe

The *dmo* gene is derived from the bacterium *Stenotrophomonas maltophilia* (Palleroni and Bradbury, 1993). *S. maltophilia* is ubiquitous in the environment and is found associated with the rhizosphere of plants. *S. maltophilia* can be found in a variety of foods and feeds, and is widespread in the home environment (Berg et al., 1999; Denton and Kerr, 1998; Echemendia, 2010). Exposure to *S. maltophilia* is incidental to its presence in food. It has been isolated from "ready to eat" salads, vegetables, frozen fish, milk, and poultry (Qureshi et al., 2005; Ryan et al., 2009). *S. maltophilia* can be found in healthy individuals without causing any harm to human health (Denton et al., 1998) and infections caused by *S. maltophilia* are extremely uncommon (Cunha, 2010). Strains have been found in the transient flora of hospitalized patients as a commensal organism (Echemendia, 2010) and, similar to the indigenous bacteria of the gastrointestinal tract, *S. maltophilia* can be an opportunistic pathogen (Berg, 1996). As such, *S. maltophilia* is of low virulence in immuno-compromised patients where a series of risk factors (severe debilitation, the presence of indwelling devices such as ventilator tubes or catheters, for prolonged periods of time and prolonged courses of antibiotics) must occur for colonization by *S. maltophilia* in humans (Ryan et al., 2009). Therefore, infections by *S. maltophilia* almost exclusively occur in hospital settings, in which case they are only present in a minimal percentage of infections (Ryan et al., 2009). Finally, *S. maltophilia* has not been reported to be source of allergens.

The ubiquitous presence of *S. maltophilia* in the environment, the presence in healthy individuals without causing infections, the incidental presence in foods without any adverse safety reports, and the lack of reported allergenicity establishes the safety of the donor organism.

V.E.1.2. MON 88701 DMO Protein Belongs to a Common Class of Mono-Oxygenases

MON 88701 DMO is classified as an oxygenase. Oxygenases are enzymes that incorporate one or two oxygen atoms into substrates and are widely distributed in many universal metabolic pathways (Harayama et al., 1992). Within this large enzymatic class are mono-oxygenases that incorporate a single oxygen atom as a hydroxyl group with the concomitant production of water and oxidation of NAD(P)H (Harayama et al., 1992). Non-heme iron oxygenases, where iron is involved in the catalytic site, are an important class of oxygenases. Within this class are Rieske oxygenases, which contain a Rieske iron-sulfur [2Fe-2S] cluster. All Rieske non-heme iron oxygenases contain two catalytic domains, a non-heme iron domain (nh-Fe) that is a site of oxygen activation, and a Rieske [2Fe-2S] domain (Ferraro et al., 2005). MON 88701 DMO belongs to this class of oxygenases which are found in diverse phyla ranging from bacteria to plants (Ferraro et al., 2005; Schmidt and Shaw, 2001).

As discussed previously, the crystal structure of a DMO has been solved (D'Ordine et al., 2009; Dumitru et al., 2009). The crystallography results demonstrated that, similar to all Rieske non-heme iron oxygenases, DMO contains two catalytically important and highly conserved domains; a mononuclear non-heme iron domain (nh-Fe) that is a site of oxygen activation, and a Rieske [2Fe-2S] domain (D'Ordine et al., 2009; Dumitru et al., 2009; Ferraro et al., 2005). The amino acids binding the non-heme iron and those that constitute the Rieske [2Fe-2S] domain in the DMO protein are also highly conserved in these plant proteins, as is their spatial orientation (D'Ordine et al., 2009; Ferraro et al., 2005). Rieske domains are ubiquitous in numerous bacterial and plant proteins like the iron-sulfur protein of the cytochrome bc1 complex, chloroplast cytochrome b6/f complex, and choline mono-oxygenases (Breyton, 2000; Darrouzet et al., 2004; Gray et al., 2004; Hibino et al., 2002; Rathinasabapathi et al., 1997; Russell et al., 1998). The presence of two conserved domains, a Rieske [2Fe-2S] domain and a mononuclear iron domain, suggests that all Rieske type non-heme iron oxygenases share the same reaction mechanism, by which the Rieske domain transfers electrons from the ferredoxin to the mononuclear iron to allow catalysis (Chakraborty et al., 2005; Dumitru et al., 2009; Ferraro et al., 2005). The structure and mechanistic homologies are further evidence of the evolutionary relatedness of all Rieske non-heme iron oxygenases to each other (Nam et al., 2001; Rosche et al., 1997; Werlen et al., 1996). Additionally, a FASTA alignment search of publicly available databases using the MON 88701 DMO protein sequence as a query yielded homologous sequences from many different species, predominantly bacteria, with amino acid sequence identity ranging up to approximately 42%. Alignments of MON 88701 DMO with plant proteins revealed homologous oxygenases present in crops such as canola (*Brassica napus*), corn (*Zea mays*), pea (*Pisum sativum*), rice (*Orysa sativa*), and soy (*Glycine max*), which were determined to have sequence identities up to approximately 27%. The highest homology was observed to proteins that are involved in chlorophyll metabolism. Chlorophyllide A oxygenase (Accession number: ACG42449) is Rieske-type oxygenase that is required for the formation of chlorophyll *b*, which is present in all plants (Tanaka et al., 1998). Pheophorbide A oxygenase (Accession number: ABD60316) is also a Rieske-type oxygenase that plays a key role in the overall regulation of chlorophyll degradation in plants (Rodoni et al., 1997). Pheophorbide A oxygenase is constitutively present in all green tissues and, at slightly lower levels, in etiolated and non-photosynthetic tissues including seeds (Yang et al., 2004). As a Rieske-type oxygenase, Pheophorbide A oxygenase is expected to have high degree of secondary and tertiary structure homology to similar structural elements in DMO as described above. The presence of these conserved structural domains in these plant proteins is further evidence that exposure to a structural homolog of MON 88701 DMO has occurred through consumption of these crops.

Therefore, MON 88701 DMO shares sequence identity and many catalytic domain structural similarities with a wide variety of oxygenases present in bacteria and plants currently widely prevalent in the environment and consumed, establishing that animals and humans are extensively exposed to these types of enzymes.

V.E.1.3. DMO Catalyzes a Specific Enzyme Reaction

DMO converts dicamba to DCSA. This demethylation is very specific to dicamba, where both the carboxylate moiety and the chlorine atoms help position the substrate at the active site of the enzyme (D'Ordine et al., 2009; Dumitru et al., 2009). Crystallography studies of the substrate in the active site demonstrated that these chlorines function as steric "handles" that position the substrate in the proper orientation in the binding pocket (Dumitru et al., 2009). Potential substrates abundant in cotton (o-anisic acid, vanillic acid, syringic acid, ferulic acid and sinapic acid) that are structurally similar to dicamba, were not metabolized by an *E. coli*-produced N-terminal histidine DMO. In addition, *E. coli*-produced MON 88701 DMO did not metabolize o-anisic acid, the endogenous compound that has the greatest structural similarity to dicamba. These laboratory tests indicate that DMO, including MON 88701 DMO protein, is specific for dicamba (Section V.A.1.2). Given the limited amount of chlorinated metabolites with structures similar to dicamba in plants and other eukaryotes (Wishart, 2010; Wishart et al., 2009), it is unlikely that MON 88701 DMO will catalyze the conversion of other endogenous substrates. Therefore, the activity of the enzyme is specific for dicamba while it maintains many structural properties common to oxygenases that are ubiquitous to all organisms with a history of safe consumption.

V.E.2. PAT (*bar*) Donor Organism, History of Safe Use, and Specificity

The safety of PAT (*bar*) protein is established in the scientific literature (Hérouet et al., 2005) and by the tolerance exemption set by the EPA (U.S. EPA, 1997). In addition, the safety of PAT proteins, including the PAT (*bar*) protein produced in MON 88701, has been assessed extensively by regulatory agencies in 11 different countries for more than 38 biotechnology-derived events in eight different species (ILSI-CERA, 2011). The PAT (*bar*) protein expressed in MON 88701 has the same functional activity as the PAT proteins in all commercially available products that provide glufosinate tolerance in several crops, including cotton, corn, soybean, and canola. The lack of any documented reports of adverse effects of glufosinate tolerant crops since their introduction in 1995 (Duke and Powles, 2009) further demonstrates the safety of PAT (*bar*) protein.

Numerous factors have been considered in the safety assessment of PAT (*bar*), which include, but are not limited to, donor organism safety, the history of safe use, and PAT protein specificity.

V.E.2.1. The *bar* Donor Organism is Safe

S. hygroscopicus is a saprophytic, soil-borne bacterium with no known safety issues. *Streptomyces* species are widespread in the environment and present no known allergenic or toxicity issues (Kämpfer, 2006; Kutzner, 1981) though human exposure is quite common (Goodfellow and Williams, 1983). *S. hygroscopicus* is not considered pathogenic to plants, humans or other animals (Cross, 1989; Goodfellow and Williams, 1983; Locci, 1989). The history of safe use of *S. hygroscopicus* is discussed previously (Hérouet et al., 2005), and this organism has been extensively reviewed during the

deregulation of several glufosinate-tolerant events with no safety or allergenicity issues identified.

The ubiquitous presence of *S. hygroscopicus* in the environment, the widespread human exposure without any adverse safety or allergenicity reports, and the successive reviews resulting from the deregulation of several glufosinate-tolerant events with no safety or allergenicity issues identified establishes the safety of the donor organism.

V.E.2.2. PAT Protein has a History of Safe Use

The PAT (*bar*) protein expressed in MON 88701 is identical to the wild-type protein produced in *S. hygroscopicus* and is analogous to the PAT proteins in commercially available glufosinate-tolerant products in several crops including cotton, corn, soybean, and canola. Based on studies characterizing the kinetic and chemical mechanisms of PAT proteins (Wehrmann et al., 1996), OECD recognizes PAT proteins produced from different genes to be equivalent with regard to function and safety (OECD, 1999b).

The safety of PAT protein present in biotechnology-derived crops has been extensively assessed (ILSI-CERA, 2011) and in 1997 a tolerance exemption was issued for PAT proteins by U.S. EPA (U.S. EPA, 1997). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, lack of significant homology to known toxins and known allergens, and lack of toxicity in an acute oral mouse gavage study. Numerous glufosinate-tolerant products including those in corn, soy, canola, sugarbeet and rice have been reviewed by the USDA and FDA with no concerns identified. Further, a comprehensive study on the safety of PAT proteins present in biotechnology-derived crops (Hérouet et al., 2005) demonstrated structural similarity only with other acetyltransferases known to not cause adverse effects after consumption, lack of sequence homology to know allergens and toxins, lack of glycosylation sites, rapid degradation in gastric and intestinal fluids, and no adverse effects in mice treated with high doses of PAT proteins. Hérouet et al. concluded that there is a reasonable certainty of no harm resulting from the inclusion of PAT proteins in human food or animal feed (2005).

The history of safe use of PAT is supported by the lack of any documented reports of adverse effects related to this protein since the introduction of glufosinate-tolerant crops in 1995 (Duke and Powles, 2009). Since then, approvals have been issued by regulatory agencies of 11 different countries for the environmental release of greater than 38 transformation events, including 8 different species of plants expressing the PAT protein (ILSI-CERA, 2011).

V.E.2.3. PAT (*bar*) Catalyzes a Specific Enzyme Reaction

The mode-of-action for PAT protein has been extensively assessed, as numerous glufosinate-tolerant products, including those in corn, soy, canola, sugarbeet, and rice, have been reviewed by the FDA and several other regulatory agencies (ILSI-CERA, 2011; OECD, 1999b; 2002a). PAT, including the PAT (*bar*) protein produced in MON 88701, is an enzyme classified as an acetyltransferase which acetylates glufosinate

to produce non-herbicidal N-acetyl glufosinate. Glufosinate is a racemic mixture of the D- and L- forms of phosphinothricin. The herbicidal activity of glufosinate results from the binding of L-phosphinothricin to glutamine synthetase (OECD, 1999b; 2002a). Glutamine synthetase is responsible for the assimilation of ammonia generated during photorespiration. The binding of L-phosphinothricin to glutamine synthetase results in the inactivation of glutamine synthetase and a subsequent toxic build-up of ammonia within the plant, resulting in death of the plant (Manderscheid and Wild, 1986; OECD, 1999b; 2002a; Wild and Manderscheid, 1984).

The PAT (*bar*) protein produced in MON 88701 acetylates the free amine group of L-phosphinothricin form of glufosinate to produce non-herbicidal N-acetyl glufosinate. The acetylated glufosinate is unable to bind to glutamine synthetase and therefore does not disrupt photorespiration and avoids the build-up of ammonia. Therefore, the production of PAT (*bar*) protein in MON 88701 confers glufosinate herbicide tolerance through this mechanism.

The PAT proteins, including PAT (*bar*), are highly specific for glufosinate in the presence of acetyl-CoA ([redacted] et al., 1987; Wehrmann et al., 1996). While the herbicidal activity of glufosinate comes from the L-amino acid form, other L-amino acids are unable to be acetylated by PAT protein and competition assays containing glufosinate, high concentrations of other amino acids and PAT showed no inhibition of glufosinate acetylation (Wehrmann et al., 1996). Furthermore, L-glutamate, an analogue of glufosinate, also showed no inhibition of glufosinate acetylation in competition assays (Wehrmann et al., 1996). In addition, the PAT (*bar*) protein has more than 30-fold higher affinity towards L-phosphinothricin over other plant analogues ([redacted] et al., 1987). Thus, the PAT (*bar*) protein has high substrate specificity for L-phosphinothricin, the herbicidal component of glufosinate, and is unlikely to affect the metabolic system of MON 88701 cotton. Numerous glufosinate-tolerant products, including those in corn, soy, canola, sugarbeet, and rice have been reviewed with no concerns identified (ILSI-CERA, 2011).

V.E.3. MON 88701 DMO and PAT (*bar*) Proteins in MON 88701 are Not Homologous to Known Allergens or Toxins

Bioinformatics analyses were performed to assess the allergenic potential, toxicity, or biological activity of MON 88701 DMO and PAT (*bar*). The analysis demonstrated that neither protein shares amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which could have adverse effects to human or animal health (Section V.D).

V.E.4. MON 88701 DMO and PAT (*bar*) Proteins in MON 88701 are Labile in *in vitro* Digestion Assays

MON 88701 DMO and PAT (*bar*) were readily digestible in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Rapid degradation of the MON 88701 DMO and PAT (*bar*) proteins in SGF and SIF makes it highly unlikely that either protein would

be absorbed in the small intestine and have any adverse effects on human or animal health.

V.E.5. MON 88701 DMO and PAT (*bar*) Proteins in MON 88701 are Not Acutely Toxic

Acute oral toxicology studies were conducted with MON 88701 DMO and PAT (*bar*) proteins individually. Results indicate that neither MON 88701 DMO or PAT (*bar*) caused any adverse effects in mice, with No Observable Adverse Effect Levels (NOAELs) for MON 88701 DMO at 283 mg/ kg bw and PAT (*bar*) at 1086 mg/kg bw, respectively, the highest doses tested.

V.E.6. Human and Animal Exposure to the MON 88701 DMO and PAT (*bar*) Proteins

Cottonseed is not consumed by humans because the majority of commercial cotton varieties contain the anti-nutrients gossypol and cyclopropenoid fatty acids. The primary human food currently produced from cottonseed is refined, bleached, and deodorized (RBD) oil, and to a smaller extent, linters. RBD oil contains undetectable amounts of protein ([redacted] and [redacted], 1979); therefore, oil produced from MON 88701 will contain extremely low levels of MON 88701 DMO and PAT (*bar*) proteins. Linters are an industrial by-product of ginning, and can be consumed as a highly processed product composed of nearly pure (*i.e.*, >99%) cellulose (NCPA, 2002; Nida et al., 1996). Cottonseed RBD oil and linters are processed fractions that contain undetectable or negligible amounts of protein there is minimal, if any, dietary exposure to MON 88701 DMO and PAT (*bar*) proteins from consumption of foods derived from MON 88701. Therefore, MOE values were not calculated for the MON 88701 DMO or PAT (*bar*) proteins. Furthermore, the safety of PAT (*bar*) has been extensively assessed (Hérouet et al., 2005), several glufosinate-tolerant crops that produce PAT proteins have been reviewed by FDA and other regulatory agencies (ILSI-CERA, 2011) and in 1997 a tolerance exemption was issued for PAT proteins by U.S. EPA (1997).

Estimated exposure of MON 88701 DMO and PAT (*bar*) proteins in animal feed were evaluated by calculating an estimate of daily dietary intake (DDI) for dairy cows. Exposure was calculated for the worst-case scenario, which assumes: 1) the source of cottonseed in the diet is cottonseed meal; 2) cottonseed meal is only derived from MON 88701 and contains no other cottonseed sources; 3) the protein expression level is the maximum expression level measured for each protein; and 4) no loss of protein due to heat. The maximum daily amount of MON 88701 DMO or PAT (*bar*) proteins consumed from MON 88701 would be for the dairy cow and would be 0.00043 g/kg of body weight for MON 88701 DMO and 0.000124 g/kg of body weight for PAT (*bar*). These values represent 0.007 and 0.002% of protein consumed, respectively. These very small levels of exposure of animals to MON 88701 DMO and PAT (*bar*) in their feed, in addition to the above mentioned safety data for both MON 88701 DMO and PAT (*bar*), support the conclusion that there is no risk to animal health when MON 88701 DMO or PAT (*bar*) are present in their diets.

V.F. MON 88701 DMO and PAT (*bar*) Protein Characterization and Safety Conclusion

MON 88701 DMO is a Rieske-type mono-oxygenase that catalyzes the O-demethylation of the herbicide dicamba and has homologs in bacteria and plants that share many of the typical structural and functional characteristics of these types of oxygenases, while maintaining specificity for its substrate. The physicochemical characteristics of the MON 88701 DMO protein were determined and equivalence between MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins was demonstrated. This equivalence justifies the use of the *E. coli*-produced MON 88701 DMO as a test substances in the protein safety studies. Expression studies using ELISA demonstrated that MON 88701 DMO was expressed at levels ranging from <LOD to 410 µg/g dw, representing a low percentage of the total protein. An assessment of the allergenic potential of the MON 88701 DMO protein supports the conclusion that the MON 88701 DMO protein does not pose a significant allergenic risk. In addition, the donor organism for the MON 88701 DMO coding sequence, *S. maltophilia*, is ubiquitous in the environment and is not commonly known for human or animal pathogenicity or allergenicity. The MON 88701 DMO protein lacks structural similarity to allergens, toxins or other proteins known to have adverse effects on mammals. The MON 88701 DMO protein is rapidly digested in simulated digestive fluids and demonstrates no oral toxicity in mice at the level tested. Based on the above information, the consumption of the MON 88701 DMO protein from MON 88701 or its progeny is considered safe for humans and animals

PAT (*bar*) protein is an acetyltransferase that catalyzes the acetylation of the herbicide glufosinate. The PAT (*bar*) protein expressed in MON 88701 is analogous to the PAT proteins in all commercially available products that provide glufosinate tolerance in several crops including cotton, corn, soybean, and canola. PAT proteins, including the PAT (*bar*) protein isolated from MON 88701 have been previously characterized, and the safety of crops expressing these proteins has been well established. The data and information provided in this section further confirms the food and feed safety of the PAT (*bar*) protein in MON 88701. The physicochemical characteristics of the PAT (*bar*) protein were determined and equivalence between MON 88701-produced and *E. coli*-produced PAT (*bar*) proteins was demonstrated. This equivalence justifies the use of the *E. coli*-produced PAT (*bar*) as a test substance in the protein safety studies. Expression studies using ELISA demonstrated that MON 88701-produced PAT (*bar*) was expressed at levels ranging from <LOD to 10 µg/g dw, representing a low percentage of the total protein. An assessment of the allergenic potential of the PAT (*bar*) protein supports the conclusion that the PAT (*bar*) protein does not pose a significant allergenic risk. In addition, the donor organism for the PAT (*bar*) coding sequence, *S. hygroscopicus*, is ubiquitous in the environment and is not commonly known for human or animal pathogenicity, or allergenicity. The PAT (*bar*) protein lacks structural similarity to allergens, toxins or other proteins known to have adverse effects on mammals. The PAT (*bar*) protein is rapidly digested in simulated digestive fluids and demonstrates no oral toxicity in mice at the level tested. Based on the above information, the consumption of the PAT (*bar*) protein from MON 88701 or its progeny is considered safe for humans and animals.

The protein safety data presented herein support the conclusion that food and feed products containing MON 88701 or derived from MON 88701 are as safe as cotton products currently on the market for human and animal consumption.

VI. COMPOSITIONAL ASSESSMENT OF MON 88701

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

A recent review of compositional assessments conducted according to OECD guidelines that encompassed a total of seven biotechnology-derived crop varieties, nine countries and eleven growing seasons concluded that incorporation of biotechnology-derived agronomic traits has had little impact on natural variation in crop composition. Most compositional variation is attributable to growing region, agronomic practices and genetic background (Harrigan et al., 2010). Compositional quality, therefore, implies a very broad range of endogenous levels of individual constituents. Numerous scientific publications have further documented the extensive variability in the concentrations of crop nutrients and anti-nutrients that reflect the influence of environmental and genetic factors as well as extensive conventional breeding efforts to improve nutrition, agronomics and yield (Reynolds et al., 2005). This observation extends to publications specific to cotton (Berberich et al., 1996; Hamilton et al., 2004; Nida et al., 1996).

Compositional equivalence between biotechnology-derived and conventional crops supports an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 2002b). The OECD consensus document on considerations for new varieties of cotton emphasize quantitative measurements of key nutrients and known anti-nutrients (OECD, 2009). This is based on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential safety and nutritional concerns (*e.g.*, anti-nutritional). Levels of the components in the seed of the biotechnology-derived crop are compared to: 1) corresponding levels in a conventional comparator, the genetically similar conventional line, grown concurrently, under the same field conditions; and 2) natural ranges generated from an evaluation of commercial reference varieties grown concurrently and from data published in the scientific literature. The comparison to data published in the literature places any potential differences between the assessed crop and its comparator in the context of the well-documented variation in the concentrations of crop nutrients and anti-nutrients.

This section provides analyses of concentrations of key nutrients and anti-nutrients of cottonseed from MON 88701 treated with both dicamba and glufosinate compared to the conventional control grown and harvested under the same conditions, as appropriate. The analyses of concentrations of key nutrients and anti-nutrients of cottonseed from MON 88701 that was not treated with either dicamba or glufosinate are presented in Appendix E as supplemental information. In addition, conventional commercial cotton reference varieties were included in the composition analyses to establish a range of natural variability for each analyte, defined by a 99% tolerance interval. The production of materials for the compositional analyses used field designs to allow accurate assessments of compositional characteristics over a range of environmental conditions

under which MON 88701 is expected to be grown. The field trial design parameters included a sufficient number of trial sites to allow adequate exposure to the variety of conditions cotton plants typically encounter in nature. Field sites were replicated with an adequate number of plants sampled, and the methods of analysis were sufficiently sensitive and specific to detect variations in the components measured to allow statistically rigorous analyses. The information provided in this section also addresses the relevant factors in Codex Plant Guidelines, Section 4, paragraphs 44 and 45 for compositional analyses (Codex Alimentarius, 2009).

VI.A. Compositional Equivalence of MON 88701 Cottonseed to Conventional Cotton

Compositional analyses comparing MON 88701 treated with dicamba and glufosinate herbicides to the conventional control variety (Coker 130) and conventional commercial reference varieties demonstrated that MON 88701 is compositionally equivalent to conventional cotton. Samples of acid-delinted cottonseed were collected from MON 88701 and the conventional control grown in a 2010 U.S. field production. Nine unique conventional cotton varieties, known as reference substances, were included across all sites of the field production with four varieties per site to provide data on natural variability of each compositional component analyzed. The field production was conducted at eight sites: Arkansas (ARTI), Georgia (GACH), Kansas (KSLA), Louisiana (LACH), North Carolina (NCBD), New Mexico (NMLC), South Carolina (SCEK) and, Texas (TXPL). The sites were planted in a randomized complete block design with four blocks per site. All cotton plants, including MON 88701, the conventional control, and the reference varieties, were grown under normal agronomic field conditions for their respective geographic regions, including maintenance pesticides as needed. In addition, MON 88701 plots were treated at the 3-5 leaf stage with glufosinate herbicide at the label rate (0.5 lbs a.i./acre), and at the 6-10 leaf stage with dicamba herbicide at the label rate (0.5 lbs a.e./acre).

Compositional analyses were conducted to assess whether levels of key nutrients and anti-nutrients in MON 88701 were equivalent to levels in the conventional control and comparable to the composition of conventional commercial reference varieties. A description of nutrients and anti-nutrients present in cotton is provided in the OECD consensus document on compositional considerations for cottonseed (OECD, 2009). Nutrients assessed in this analysis included proximates (ash, calories and carbohydrates by calculation, fat, moisture, and protein), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber (CF), total dietary fiber (TDF), amino acids (AA, 18 components), fatty acids (FA, C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), and vitamin E. Methods used in the assessments of nutrients and anti-nutrients are found in Appendix E. In all, 65 different analytical components were measured. Due to statistical constraints, in order to proceed with the statistical analysis of any component in this study, at least 50% of the observed values for that analyte needed to be greater than the assay limit of quantitation (LOQ). Of the 65 components measured, 13 had more than 50% of the observations below the assay LOQ and were excluded from statistical analysis. Therefore, 52 components were statistically assessed using a mixed-model analysis of variance method.

Values for all components were expressed on a dry weight basis with the exception of moisture, expressed as percent fresh weight, and fatty acids, expressed as percent of total FA.

For MON 88701, nine sets of statistical comparisons to the conventional control were conducted. One comparison was based on compositional data combined across all eight field sites (the combined-site analysis) and eight separate comparisons to the conventional control were conducted on data from each of the eight individual field sites. Statistically significant differences were identified at a 5% level of significance ($p<0.05$). Compositional data from the conventional commercial reference varieties, grown concurrently in the same trial as MON 88701 and the conventional control, Coker 130, were combined across all sites and used to calculate a 99% tolerance interval for each component to define the natural variability in cotton varieties that have a history of safe consumption.

For the combined-site analysis, statistically significant differences ($p < 0.05$) in nutrient and anti-nutrient components were evaluated further using considerations relevant to the safety and nutritional quality of MON 88701 when compared to the conventional control. The evaluation included: 1) the relative magnitude of the significant difference in the mean values of nutrient and anti-nutrient components of MON 88701 compared to the conventional control; 2) whether the MON 88701 component mean values were within the range of natural variability of that component as represented by the 99% tolerance interval of commercial conventional reference varieties grown concurrently in the same trial; 3) analyses of the reproducibility of the significant combined-site component differences at individual sites; and 4) assessing the combined-site statistically significant differences and reproducible individual site significant differences within the context of natural variability of commercial cottonseed composition published in the scientific literature and/or in the International Life Sciences Institute Crop Composition Database (ILSI, 2011) (See Table VI-4). Statistical summaries of nutrients and anti-nutrients for individual sites are found in Appendix E.

This analysis provides a comprehensive comparative assessment of the levels of key nutrients and anti-nutrients in cottonseed of MON 88701 and the conventional control discussed in the context of natural variability in composition of commercial cotton. Results of the comparison indicate that the composition of the cottonseed of MON 88701 is equivalent to that of conventional cotton.

Compositional results from MON 88701 plots treated with dicamba and glufosinate label rates are summarized in the following subsections. Similar results were obtained for MON 88701 plots that were not treated with either dicamba or glufosinate, which are provided as additional information in Appendix E.

VI.A.1 Nutrient Levels in Cottonseed

In the combined-site analysis of nutrient levels in cottonseed, the following components had no statistically significant differences ($p<0.05$) in mean values between MON 88701 and the conventional control: one proximate (protein), one type of fiber (crude fiber), 15

amino acids (alanine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine), seven fatty acids (16:0 palmitic acid, 16:1 palmitoleic acid, 18:0 stearic acid, 18:1 oleic acid, 18:3 linolenic acid, 20:0 arachidic acid, and 22:0 behenic acid), and four minerals (copper, iron, phosphorus, and sodium) (Table VI-1 and VI-2).

The components that had significant differences in mean values between MON 88701 and the conventional control in the combined-site analysis were: five proximates (ash, calories, carbohydrates, moisture, and total fat), three types of fiber (ADF, NDF, and TDF), three amino acids (arginine, methionine and proline), two fatty acids (14:0 myristic acid and 18:2 linoleic acid), five minerals (calcium, magnesium, manganese, potassium, and zinc) and vitamin E (Table VI-1).

The statistically significant differences in nutrients were further evaluated using the four previously described considerations relevant to the safety and nutritional quality of MON 88701 when compared to the conventional control:

1) All nutrient component differences observed in the combined-site statistical analysis, whether reflecting increased or decreased MON 88701 mean values with respect to the conventional control, were 14.09% or less. The relative magnitudes of the differences were: 0.66 to 5.00% for proximates, 4.08 to 5.72% for fibers, 2.61 to 4.82% for amino acids, 0.69 to 2.69% for fatty acids, 4.94 to 14.09% for minerals and 6.70% for vitamin E.

2) With the exception of methionine, mean values for all significantly different nutrient components from the combined-site analysis of MON 88701 were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial.

3) Assessment of the reproducibility of the combined-site differences at the eight individual sites showed significant differences for: NDF, methionine, proline and 18:2 linoleic acid at one site; carbohydrates, total fat, ADF, manganese and zinc at two sites; TDF, arginine, 14:0 myristic acid, potassium, and vitamin E at three sites; magnesium at four sites, ash at six sites and calcium at seven sites. Moisture and calories were not affected at any site. With the exception of methionine, arginine, and zinc, all individual site mean values of MON 88701 for all nutrient components with significant differences were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial.

4) All combined-site mean values and individual mean values of MON 88701 for all nutrient components, including those that were significantly different, were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Five of the 19 cottonseed nutrient statistically significant differences between MON 88701 and the conventional control that were observed in the combined-site data analysis were attributable to small differences in proximates (ash, carbohydrates, total fat expressed as % dw, calories expressed as Kcal/100g dw, and moisture expressed as % fw). For ash, calories, and total fat the relative magnitude of the differences between the mean value for MON 88701 and the conventional control were all small increases (5.00% for ash, 0.66% for calories and 3.71% for total fat). The differences for carbohydrates and moisture between the mean value for MON 88701 and the conventional control were both small decreases (2.60% for carbohydrates and 4.51% for moisture). All of the nutrient mean values for MON 88701 observed in the combined-site analysis for proximates were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Except for ash, significant differences for most proximate mean values between MON 88701 and the conventional control were not consistently observed among individual sites. There were no significant differences at any of the individual sites for calories or moisture. Total fat was increased at two sites ranging from 6.74 to 8.46% and carbohydrates were decreased at two sites, with decreases ranging from 4.33 to 5.08%. Although ash was increased in MON 88701 when compared to the conventional control at six sites, increases ranged from 4.95 to 11.50%, which was less than the variability for the control samples (range 3.46 to 4.29, a relative difference of 24.0%, Table VI-1). Overall, observed differences in proximate values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because the magnitudes of combined-site differences ranged only from 0.66% to 5.00%, most were not consistently reproduced across the individual sites, and the mean MON 88701 combined-site values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial, and were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Three of the 19 cottonseed nutrient statistically significant differences between MON 88701 and the conventional control observed in the combined-site data analysis were attributable to small differences in fiber (ADF, NDF, and TDF all expressed as % dw). All relative magnitudes of the differences for fiber between the mean values for MON 88701 and the conventional control were small decreases (4.94% for ADF, 5.72% for NDF and 4.08% for TDF). All of the nutrient mean values for MON 88701 observed in the combined-site analysis for fiber were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for fiber mean values between MON 88701 and the conventional control were not consistently observed among individual sites. TDF and ADF were decreased at three and two sites, respectively, with decreases ranging from 4.55 to 8.15% for TDF and 9.27 to 9.86% for ADF. NDF was significantly different at one site with a small decrease of 7.40%. Overall, observed differences in fiber values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were small, not consistently reproduced across the individual sites, and the mean MON 88701 combined-site values

were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial, and were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Three other combined-site nutrient statistically significant differences between MON 88701 and the conventional control observed in the combined-site analysis were attributed to small differences in amino acids (arginine, methionine, and proline; expressed as % dw). For both arginine and proline, the relative magnitude of the differences between the mean values for MON 88701 and the conventional control were small decreases (3.80% for arginine and 2.61% for proline). Methionine was increased 4.82% when MON 88701 was compared to the conventional control. With the exception of methionine, the nutrient mean values for MON 88701 observed in the combined-site analysis for amino acids were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. The combined-site mean value for methionine was within the context of natural variation of methionine found in commercial cotton as published in the scientific literature or as found in the ILSI Crop Composition Database (ILSI, 2011). Significant differences for amino acid mean values between MON 88701 and the conventional control were not consistently observed at all eight individual sites. Arginine and proline were decreased at three sites and one site, respectively, with decreases ranging from 6.10 to 8.35% for arginine and 6.16% for proline. Methionine was increased 12.03% at only one site. Overall, observed differences in amino acid values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were small in magnitude, not consistently reproduced across the individual sites, and with the exception of methionine, the mean MON 88701 combined-site values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. In addition, all MON 88701 amino acid values were within the context of the natural variability of commercial cotton composition as published in the scientific literature or available in the ILSI Crop Composition Database (ILSI, 2011).

Two of the combined-site nutrient statistically significant differences between MON 88701 and the conventional control were attributed to the fatty acids 14:0 myristic acid and 18:2 linoleic acid (expressed as % total FA). The relative magnitudes of the differences between the mean fatty acid values for MON 88701 and the conventional control in the combined-site analysis were small decreases (2.69% for 14:0 myristic acid and 0.69% for 18:2 linoleic acid). The nutrient mean values for MON 88701 observed in the combined-site analysis for both 14:0 myristic acid and 18:2 linoleic acid were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for fatty acid mean values between MON 88701 and the conventional control were not consistently observed among individual sites. 14:0 myristic acid was decreased at three sites while 18:2 linoleic acid was decreased at one site with differences ranging from 4.43 to 8.36% for 14:0 myristic acid and 1.93% for 18:2 linoleic acid. Overall, observed differences in fatty acid values between MON 88701 and the conventional control were not considered to be meaningful

from a food and feed safety and nutritional perspective because they were small, not consistently reproduced across the individual sites, and the mean MON 88701 values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Five of the 19 cottonseed nutrient statistically significant differences between MON 88701 and the conventional control observed in the combined-site analysis were attributed to small differences in minerals (calcium, magnesium, and potassium expressed as % dw and manganese and zinc expressed as mg/kg dw). For calcium, magnesium, potassium, and manganese, the relative magnitudes of the differences between the mean values for MON 88701 and the conventional control were increases of 14.09% for calcium, 5.63% for magnesium, 9.20% for manganese, and 4.94% for potassium. The relative magnitude of the difference for zinc between the mean value for MON 88701 and the conventional control was a decrease of 6.39%. All of the nutrient mean values for MON 88701 observed in the combined-site analysis for minerals were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Except for calcium, significant differences for mineral mean values between MON 88701 and the conventional control were not consistently observed among individual sites. Calcium was significantly different at seven sites, with increases ranging from 6.92 to 22.70%; this was less than the variability observed for the control samples (range 0.091 to 0.18, a relative difference of 97.8%, Table VI-1).

Magnesium, potassium, and manganese were significantly different at four, three, and two sites, respectively, with increases ranging from 5.54 to 9.36% for magnesium, 8.01 to 16.37% for potassium and from 16.52 to 20.59% for manganese. Zinc was significantly different at two sites, with decreases ranging from 7.68 to 17.66%. Overall, observed differences in mineral values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were small in magnitude, not consistently reproduced across the individual sites (with the exception of calcium), and the mean MON 88701 combined-site values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

One other nutrient difference observed in the combined-site analysis between MON 88701 and the conventional control was attributed to vitamin E (expressed as mg/kg dw). The relative magnitude of the difference between the mean vitamin E value for MON 88701 and the conventional control in the combined-site analysis was a small increase of 6.70%. The nutrient mean value for MON 88701 observed in the combined-site analysis for vitamin E was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for vitamin E mean values between MON 88701 and the conventional control were not consistently observed among individual sites, with

significant increases ranging from 7.78 to 13.28% observed at three sites. Overall, the observed difference in the vitamin E values between MON 88701 and the conventional control in the combined-site analysis were not considered to be meaningful from a food and feed safety and nutritional perspective because they were 13.28% or less, not consistently reproduced across the individual sites, and the mean MON 88701 values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial, and were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

In summary, except for calcium and ash, statistical analyses found no consistent differences between the levels of nutrient components in cottonseed from MON 88701 and the conventional control. Differences were observed for calcium and ash in combined-site analyses and most individual sites, but the magnitudes of differences for these nutrients were less than the variability for the control samples, and values were within the range of natural variability for cottonseed. These findings support the conclusion of compositional equivalence of MON 88701 to conventional cotton.

VI.A.2. Anti-Nutrient Levels in Cottonseed

Cottonseed was analyzed for five anti-nutrients and in the combined-site analysis the following components had no significant differences ($p<0.05$) in mean values between MON 88701 treated with dicamba and glufosinate and the conventional control: two cyclopropenoid fatty acids (malvalic and sterculic) (Table VI-3). The components that showed statistically significant differences in mean values between MON 88701 and the conventional control were: one cyclopropenoid fatty acid (dihydrosterculic), free gossypol, and total gossypol (Table VI-1).

The statistically significant differences in anti-nutrients were further evaluated using the four previously described considerations relevant to the safety and nutritional quality of MON 88701 when compared to the conventional control:

1) All anti-nutrient component differences observed in the combined-site statistical analysis, which reflected an increase in MON 88701 mean values with respect to the conventional control, were small in magnitude. The relative magnitude of the differences for dihydrosterculic acid, free gossypol, and total gossypol were 9.59%, 6.23%, and 6.75%, respectively.

2) Mean values for all significantly different anti-nutrient components from the combined-site analysis of MON 88701 were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial.

3) Assessment of the reproducibility of the combined-site differences at the eight individual sites showed significant differences for: dihydrosterculic at one site; free gossypol at two sites; and total gossypol at three sites. All individual site mean values of MON 88701 for all anti-nutrient components with significant

differences were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial.

4) All combined-site mean values of MON 88701 for all anti-nutrient components, including those that were significantly different, were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

The three cottonseed anti-nutrient differences between MON 88701 and the conventional control observed in the combined-site data analysis were attributed to small differences in one cyclopropenoid fatty acid (dihydrosterculic; expressed as % total fatty acid), free gossypol, and total gossypol (expressed as % dw). For dihydrosterculic acid, free gossypol, and total gossypol, the relative magnitude of the differences between the mean values for MON 88701 and the conventional control were increases of 9.59% for dihydrosterculic acid, 6.23% for free gossypol, and 6.75% for total gossypol. These anti-nutrient differences between MON 88701 and the conventional control observed in the combined-site analysis were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for the three anti-nutrient mean values between MON 88701 and the conventional control were not consistently observed across all eight individual sites. Dihydrosterculic acid, free gossypol, and total gossypol were significantly different at one, two, and three sites respectively, with an increase of 28.35% for dihydrosterculic acid, and increases ranging from 12.69 to 22.32% for free gossypol and 9.54 to 15.53% for total gossypol. Overall, observed differences in anti-nutrient values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were generally small, not consistently reproduced across the individual sites, and the mean MON 88701 values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

In summary, statistical analyses found no consistent statistically significant differences between the levels of anti-nutrient components in cottonseed from MON 88701 and the conventional control and mean values for anti-nutrients were within the range of natural variability for cottonseed. These findings supported the conclusion of compositional equivalence of MON 88701 to conventional cotton.

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Cottonseed Proximate (% dw)						
Ash	4.31	4.11	5.00	0.001	3.77 - 4.74	3.42, 4.65
Calories Kcal/100g	498.50	495.24	0.66	0.013	482.46 - 517.46	457.61, 527.56
Carbohydrates	44.64	45.83	-2.60	<0.001	41.40 - 48.89	40.26, 56.45
Moisture (% fw)	7.15	7.48	-4.51	0.005	5.93 - 9.67	4.79, 9.92
Total Fat	23.14	22.31	3.71	0.001	19.79 - 26.78	15.01, 28.51
Cottonseed Fiber (% dw)						
Acid Detergent Fiber	25.27	26.58	-4.94	0.002	23.26 - 27.74	22.24, 31.96
Neutral Detergent Fiber	30.73	32.59	-5.72	<0.001	25.13 - 34.42	27.03, 42.49

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Cottonseed Fiber (% dw)						
Total Dietary Fiber	39.44	41.12	-4.08	<0.001	36.91 - 42.13	34.52, 52.58
Cottonseed Amino Acid (% dw)						
Arginine	3.03	3.15	-3.80	0.002	2.33 - 3.60	2.38, 3.47
Methionine	0.40	0.38	4.82	0.026	0.35 - 0.46	0.32, 0.38
Proline	1.00	1.03	-2.61	0.037	0.82 - 1.21	0.83, 1.08
Cottonseed Fatty Acid (% Total FA)						
14:0 Myristic	0.77	0.79	-2.69	0.009	0.66 - 0.95	0.16, 1.37
18:2 Linoleic	55.77	56.15	-0.69	0.026	54.24 - 58.22	47.49, 63.18

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Cottonseed Mineral						
Calcium (% dw)	0.15	0.13	14.09	<0.001	0.10 - 0.22	0.058, 0.21
Magnesium (% dw)	0.40	0.38	5.63	<0.001	0.35 - 0.44	0.28, 0.47
Manganese (mg/kg dw)	12.81	11.73	9.20	0.001	10.18 - 14.81	9.07, 17.33
Potassium (% dw)	1.12	1.07	4.94	0.021	0.98 - 1.24	0.92, 1.21
Zinc (mg/kg dw)	37.58	40.14	-6.39	0.005	27.31 - 46.74	27.27, 44.95
Cottonseed Vitamin (mg/kg dw)						
Vitamin E	140.14	131.33	6.70	<0.001	86.23 - 179.34	41.91, 205.89
Cottonseed Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15	0.14	9.59	0.003	0.11 - 0.19	0.078, 0.25

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Cottonseed Gossypol (% dw)						
Free Gossypol	0.94	0.89	6.23	0.016	0.80 - 1.18	0.099, 1.57
Total Gossypol	1.04	0.97	6.75	<0.001	0.84 - 1.24	0.064, 1.76
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 7 Sites						
Calcium (% dw) Site ARTI	0.15	0.12	22.70	0.010	0.14 - 0.16	0.058, 0.21
Calcium (% dw) Site GACH	0.13	0.11	17.57	<0.001	0.13 - 0.13	0.058, 0.21
Calcium (% dw) Site KSLA	0.20	0.18	14.74	0.007	0.19 - 0.22	0.058, 0.21
Calcium (% dw) Site NCBD	0.15	0.14	6.92	0.007	0.14 - 0.15	0.058, 0.21
Calcium (% dw) Site NMLC	0.15	0.13	16.83	0.003	0.14 - 0.15	0.058, 0.21

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 7 Sites						
Calcium (% dw) Site SCEK	0.11	0.091	17.98	0.027	0.10 - 0.11	0.058, 0.21
Calcium (% dw) Site TXPL	0.16	0.14	15.31	<0.001	0.16 - 0.16	0.058, 0.21
Cottonseed Proximate (% dw) - 6 Sites						
Ash Site GACH	4.53	4.21	7.56	<0.001	4.45 - 4.57	3.42, 4.65
Ash Site KSLA	4.53	4.29	5.64	0.027	4.25 - 4.66	3.42, 4.65
Ash Site LACH	4.35	4.12	5.56	0.013	4.23 - 4.47	3.42, 4.65
Ash Site NCBD	4.34	4.14	4.95	0.033	4.29 - 4.40	3.42, 4.65
Ash Site SCEK	4.11	3.74	9.95	0.010	3.99 - 4.28	3.42, 4.65

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Proximate (% dw) - 6 Sites						
Ash Site TXPL	3.85	3.46	11.50	0.001	3.77 - 3.92	3.42, 4.65
Cottonseed Fatty Acid (% Total FA) - 5 Sites						
18:0 Stearic Site ARTI	2.68	2.51	6.70	0.019	2.65 - 2.72	1.98, 2.95
18:0 Stearic Site LACH	2.68	2.52	6.04	0.001	2.64 - 2.73	1.98, 2.95
18:0 Stearic Site NCBD	2.50	2.34	6.85	0.036	2.39 - 2.64	1.98, 2.95
18:0 Stearic Site NMLC	2.51	2.64	-5.13	<0.001	2.47 - 2.56	1.98, 2.95
18:0 Stearic Site TXPL	2.35	2.46	-4.67	0.006	2.30 - 2.43	1.98, 2.95
Cottonseed Mineral - 4 Sites						
Magnesium (% dw) Site GACH	0.41	0.38	6.92	<0.001	0.40 - 0.41	0.28, 0.47

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 4 Sites						
Magnesium (% dw) Site KSLA	0.43	0.40	6.85	0.002	0.41 - 0.43	0.28, 0.47
Magnesium (% dw) Site SCEK	0.39	0.36	9.36	0.005	0.37 - 0.41	0.28, 0.47
Magnesium (% dw) Site TXPL	0.35	0.34	5.54	0.003	0.35 - 0.37	0.28, 0.47
Cottonseed Fiber (% dw) - 3 Sites						
Total Dietary Fiber Site KSLA	38.32	40.14	-4.55	0.034	37.62 - 38.75	34.52, 52.58
Total Dietary Fiber Site LACH	39.82	43.35	-8.15	0.002	39.02 - 40.86	34.52, 52.58
Total Dietary Fiber Site NMLC	39.16	41.10	-4.73	0.016	37.46 - 40.44	34.52, 52.58
Cottonseed Amino Acid (% dw) - 3 Sites						
Arginine Site GACH	2.95	3.21	-8.35	0.008	2.87 - 3.02	2.38, 3.47

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Amino Acid (% dw) - 3 Sites						
Arginine Site KSLA	3.02	3.28	-7.87	0.013	2.95 - 3.10	2.38, 3.47
Arginine Site NMLC	3.48	3.71	-6.10	0.005	3.42 - 3.60	2.38, 3.47
Cottonseed Fatty Acid (% Total FA) - 3 Sites						
14:0 Myristic Site KSLA	0.68	0.72	-5.33	0.007	0.66 - 0.71	0.16, 1.37
14:0 Myristic Site NCBD	0.68	0.75	-8.36	0.002	0.66 - 0.70	0.16, 1.37
14:0 Myristic Site NMLC	0.93	0.98	-4.43	0.001	0.92 - 0.95	0.16, 1.37
Cottonseed Mineral - 3 Sites						
Potassium (% dw) Site GACH	1.21	1.12	8.01	<0.001	1.17 - 1.24	0.92, 1.21
Potassium (% dw) Site SCEK	1.13	1.02	10.88	0.042	1.11 - 1.17	0.92, 1.21

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 3 Sites						
Potassium (% dw) Site TXPL	1.01	0.87	16.37	0.004	0.98 - 1.06	0.92, 1.21
Cottonseed Vitamin (mg/kg dw) - 3 Sites						
Vitamin E Site GACH	151.03	140.12	7.78	0.025	148.34 - 154.95	41.91, 205.89
Vitamin E Site LACH	169.88	149.96	13.28	0.001	163.34 - 175.33	41.91, 205.89
Vitamin E Site TXPL	114.39	103.66	10.35	0.033	107.81 - 118.39	41.91, 205.89
Cottonseed Gossypol (% dw) - 3 Sites						
Total Gossypol Site KSLA	1.13	1.01	12.00	0.049	1.00 - 1.24	0.064, 1.76
Total Gossypol Site NMLC	0.92	0.80	15.53	0.026	0.84 - 0.97	0.064, 1.76
Total Gossypol Site SCEK	1.17	1.07	9.54	0.017	1.13 - 1.23	0.064, 1.76

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Proximate (% dw) - 2 Sites						
Carbohydrates Site SCEK	46.56	48.67	-4.33	0.031	45.10 - 47.48	40.26, 56.45
Carbohydrates Site TXPL	44.03	46.39	-5.08	0.010	42.73 - 45.99	40.26, 56.45
Total Fat Site NCBD	23.04	21.59	6.74	0.024	21.89 - 23.76	15.01, 28.51
Total Fat Site SCEK	25.65	23.65	8.46	0.019	24.23 - 26.78	15.01, 28.51
Cottonseed Fiber (% dw) - 2 Sites						
Acid Detergent Fiber Site ARTI	24.81	27.53	-9.86	0.007	24.44 - 25.20	22.24, 31.96
Acid Detergent Fiber Site LACH	25.72	28.35	-9.27	0.005	24.16 - 27.08	22.24, 31.96
Cottonseed Amino Acid (% dw) - 2 Sites						
Phenylalanine Site GACH	1.40	1.49	-5.89	0.039	1.37 - 1.43	1.12, 1.58

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Amino Acid (% dw) - 2 Sites						
Phenylalanine Site KSLA	1.44	1.53	-5.88	0.025	1.40 - 1.46	1.12, 1.58
Cottonseed Fatty Acid (% Total FA) - 2 Sites						
16:0 Palmitic Site LACH	24.48	24.04	1.81	0.014	24.37 - 24.55	16.54, 30.55
16:0 Palmitic Site SCEK	24.74	24.39	1.43	0.029	24.59 - 24.94	16.54, 30.55
16:1 Palmitoleic Site NCBD	0.46	0.48	-3.88	0.019	0.44 - 0.47	0.39, 0.70
16:1 Palmitoleic Site NMLC	0.53	0.54	-2.27	0.014	0.52 - 0.53	0.39, 0.70
18:3 Linolenic Site ARTI	0.14	0.13	11.92	0.012	0.14 - 0.15	0.060, 0.24
18:3 Linolenic Site NMLC	0.16	0.14	8.12	0.009	0.15 - 0.16	0.060, 0.24

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 2 Sites						
Iron (mg/kg dw) Site NCBD	43.21	48.04	-10.05	0.025	41.96 - 44.44	47.30, 97.12
Iron (mg/kg dw) Site TXPL	60.47	79.02	-23.47	0.039	56.94 - 66.50	47.30, 97.12
Manganese (mg/kg dw) Site GACH	13.41	11.51	16.52	0.003	12.79 - 14.14	9.07, 17.33
Manganese (mg/kg dw) Site TXPL	10.91	9.04	20.59	0.007	10.18 - 11.37	9.07, 17.33
Zinc (mg/kg dw) Site NCBD	40.79	49.54	-17.66	0.006	40.28 - 41.37	27.27, 44.95
Zinc (mg/kg dw) Site NMLC	45.63	49.43	-7.68	0.009	44.12 - 46.74	27.27, 44.95
Cottonseed Gossypol (% dw) - 2 Sites						
Free Gossypol Site KSLA	1.07	0.95	12.69	0.014	1.03 - 1.10	0.099, 1.57

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Gossypol (% dw) - 2 Sites						
Free Gossypol Site NMLC	0.85	0.69	22.32	0.011	0.83 - 0.88	0.099, 1.57
Statistical Differences Observed in One Site						
Cottonseed Proximate (% dw)						
Protein Site TXPL	29.43	28.48	3.33	0.017	29.06 - 30.14	22.30, 29.41
Cottonseed Fiber (% dw)						
Crude Fiber Site KSLA	16.43	17.67	-7.04	0.019	16.06 - 17.24	16.93, 22.68
Neutral Detergent Fiber Site TXPL	29.75	32.12	-7.40	0.006	28.74 - 30.56	27.03, 42.49
Cottonseed Amino Acid (% dw)						
Alanine Site LACH	1.07	1.03	3.73	0.030	1.00 - 1.11	0.86, 1.11
Aspartic Acid Site GACH	2.31	2.45	-6.03	0.019	2.24 - 2.36	1.94, 2.57

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Site						
Cottonseed Amino Acid (% dw)						
Glutamic Acid Site GACH	4.57	4.96	-7.95	0.010	4.35 - 4.77	3.74, 5.28
Isoleucine Site GACH	0.90	0.94	-4.21	0.034	0.90 - 0.91	0.75, 0.96
Leucine Site GACH	1.51	1.58	-4.32	0.024	1.49 - 1.54	1.25, 1.62
Lysine Site LACH	1.26	1.18	7.01	0.023	1.17 - 1.31	1.01, 1.30
Methionine Site LACH	0.42	0.38	12.03	0.013	0.37 - 0.44	0.32, 0.38
Proline Site GACH	0.98	1.05	-6.16	0.033	0.97 - 0.99	0.83, 1.08
Threonine Site GACH	0.85	0.90	-5.14	0.049	0.83 - 0.88	0.72, 0.89
Tryptophan Site SCEK	0.35	0.38	-6.70	0.023	0.33 - 0.38	0.34, 0.42

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Site						
Cottonseed Amino Acid (% dw)						
Tyrosine Site GACH	0.80	0.84	-4.30	0.037	0.79 - 0.82	0.67, 0.84
Valine Site GACH	1.21	1.26	-4.19	0.017	1.19 - 1.23	1.00, 1.28
Cottonseed Fatty Acid (% Total FA)						
18:1 Oleic Site LACH	14.70	14.29	2.89	0.021	14.48 - 15.01	11.38, 20.64
18:2 Linoleic Site LACH	55.53	56.63	-1.93	0.001	55.15 - 55.99	47.49, 63.18
20:0 Arachidic Site LACH	0.31	0.29	6.78	0.033	0.31 - 0.32	0.17, 0.38
22:0 Behenic Site ARTI	0.14	0.15	-9.92	0.008	0.13 - 0.14	0.070, 0.21
Cottonseed Mineral						
Sodium (% dw) Site KSLA	0.022	0.0080	178.30	0.020	0.019 - 0.025	0, 0.066

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Site						
Cottonseed Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid Site GACH	0.15	0.12	28.35	0.022	0.14 - 0.16	0.078, 0.25

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 was treated with dicamba and glufosinate.
[3]Mean = least-square mean.
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.31 (0.11) (3.77 - 4.74)	4.11 (0.11) (3.34 - 5.00)	0.21 (0.052) (-0.49 - 0.61)	0.094, 0.32	0.001	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	498.50 (1.65) (482.46 - 517.46)	495.24 (1.71) (487.70 - 512.65)	3.26 (1.29) (-14.30 - 18.37)	0.70, 5.82	0.013	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.64 (0.56) (41.40 - 48.89)	45.83 (0.57) (42.14 - 50.30)	-1.19 (0.32) (-5.19 - 2.45)	-1.82, -0.56	<0.001	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	7.15 (0.26) (5.93 - 9.67)	7.48 (0.27) (6.15 - 9.19)	-0.34 (0.11) (-1.82 - 0.79)	-0.56, -0.11	0.005	4.79, 9.92 (6.05 - 10.50)
Protein	27.91 (0.77) (22.71 - 31.47)	27.79 (0.77) (23.53 - 31.27)	0.13 (0.31) (-1.99 - 3.73)	-0.53, 0.78	0.685	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.14 (0.31) (19.79 - 26.78)	22.31 (0.33) (20.71 - 25.20)	0.83 (0.26) (-2.89 - 3.86)	0.32, 1.34	0.001	15.01, 28.51 (16.58 - 25.25)

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.27 (0.34) (23.26 - 27.74)	26.58 (0.35) (22.08 - 29.58)	-1.31 (0.35) (-5.42 - 1.77)	-2.06, -0.57	0.002	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	18.17 (0.37) (15.97 - 21.66)	18.54 (0.38) (16.06 - 21.70)	-0.38 (0.32) (-3.36 - 4.75)	-1.02, 0.27	0.246	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	30.73 (0.51) (25.13 - 34.42)	32.59 (0.53) (28.87 - 35.89)	-1.86 (0.41) (-6.95 - 1.16)	-2.68, -1.05	<0.001	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.44 (0.39) (36.91 - 42.13)	41.12 (0.41) (39.05 - 44.37)	-1.68 (0.36) (-5.34 - 1.09)	-2.45, -0.91	<0.001	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.06 (0.020) (0.91 - 1.14)	1.05 (0.020) (0.88 - 1.17)	0.0026 (0.0091) (-0.13 - 0.12)	-0.017, 0.022	0.775	0.86, 1.11 (0.83 - 1.22)
Arginine	3.03 (0.10) (2.33 - 3.60)	3.15 (0.10) (2.41 - 3.77)	-0.12 (0.033) (-0.47 - 0.39)	-0.19, -0.049	0.002	2.38, 3.47 (2.30 - 3.55)

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.39 (0.062) (1.94 - 2.64)	2.41 (0.062) (1.92 - 2.74)	-0.015 (0.027) (-0.29 - 0.29)	-0.072, 0.042	0.575	1.94, 2.57 (1.79 - 2.72)
Cystine	0.41 (0.0091) (0.32 - 0.47)	0.40 (0.0094) (0.31 - 0.46)	0.0096 (0.0070) (-0.063 - 0.082)	-0.0043, 0.023	0.174	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.76 (0.13) (3.80 - 5.38)	4.84 (0.14) (3.66 - 5.70)	-0.079 (0.072) (-0.78 - 0.79)	-0.23, 0.077	0.295	3.74, 5.28 (3.39 - 5.45)
Glycine	1.10 (0.020) (0.93 - 1.19)	1.09 (0.020) (0.91 - 1.20)	0.0014 (0.011) (-0.13 - 0.14)	-0.021, 0.024	0.896	0.90, 1.14 (0.85 - 1.23)
Histidine	0.74 (0.019) (0.58 - 0.85)	0.75 (0.019) (0.61 - 0.84)	-0.0014 (0.0073) (-0.062 - 0.091)	-0.017, 0.014	0.854	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.91 (0.018) (0.75 - 1.01)	0.92 (0.018) (0.77 - 1.03)	-0.0066 (0.0079) (-0.077 - 0.096)	-0.023, 0.010	0.421	0.75, 0.96 (0.72 - 1.03)

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.53 (0.032) (1.29 - 1.70)	1.54 (0.032) (1.28 - 1.69)	-0.0018 (0.013) (-0.14 - 0.16)	-0.029, 0.026	0.892	1.25, 1.62 (1.20 - 1.72)
Lysine	1.24 (0.025) (1.05 - 1.38)	1.23 (0.025) (1.06 - 1.39)	0.0069 (0.015) (-0.11 - 0.15)	-0.026, 0.039	0.658	1.01, 1.30 (0.99 - 1.44)
Methionine	0.40 (0.0079) (0.35 - 0.46)	0.38 (0.0084) (0.32 - 0.46)	0.018 (0.0081) (-0.066 - 0.12)	0.0023, 0.035	0.026	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.43 (0.039) (1.14 - 1.66)	1.46 (0.039) (1.15 - 1.66)	-0.022 (0.014) (-0.18 - 0.19)	-0.052, 0.0084	0.144	1.12, 1.58 (1.10 - 1.63)
Proline	1.00 (0.029) (0.82 - 1.21)	1.03 (0.029) (0.81 - 1.25)	-0.027 (0.012) (-0.12 - 0.10)	-0.052, -0.0018	0.037	0.83, 1.08 (0.79 - 1.17)
Serine	1.08 (0.025) (0.90 - 1.23)	1.09 (0.026) (0.86 - 1.24)	-0.0036 (0.015) (-0.18 - 0.16)	-0.035, 0.028	0.807	0.83, 1.21 (0.81 - 1.24)

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.87 (0.016) (0.74 - 0.94)	0.86 (0.016) (0.73 - 0.95)	0.0057 (0.0083) (-0.10 - 0.10)	-0.012, 0.023	0.504	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.0092) (0.33 - 0.52)	0.42 (0.0095) (0.37 - 0.52)	-0.0061 (0.0066) (-0.081 - 0.078)	-0.019, 0.0071	0.361	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.81 (0.017) (0.67 - 0.92)	0.81 (0.018) (0.67 - 0.91)	-0.0011 (0.0083) (-0.074 - 0.12)	-0.019, 0.017	0.898	0.67, 0.84 (0.63 - 0.91)
Valine	1.21 (0.027) (1.00 - 1.40)	1.23 (0.027) (1.00 - 1.40)	-0.012 (0.011) (-0.090 - 0.12)	-0.036, 0.012	0.296	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.77 (0.030) (0.66 - 0.95)	0.79 (0.031) (0.71 - 0.98)	-0.021 (0.0071) (-0.077 - 0.047)	-0.036, -0.0060	0.009	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	23.95 (0.30) (22.34 - 25.28)	23.80 (0.30) (22.69 - 25.05)	0.15 (0.076) (-0.68 - 0.76)	-0.016, 0.31	0.073	16.54, 30.55 (19.11 - 26.73)

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.50 (0.0094) (0.44 - 0.54)	0.50 (0.0094) (0.45 - 0.54)	0.0022 (0.0038) (-0.025 - 0.039)	-0.0060, 0.010	0.572	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.54 (0.058) (2.29 - 2.85)	2.47 (0.058) (2.15 - 2.76)	0.068 (0.036) (-0.16 - 0.24)	-0.0091, 0.14	0.079	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	15.10 (0.26) (14.15 - 16.45)	14.96 (0.26) (14.06 - 16.44)	0.14 (0.070) (-0.48 - 0.75)	-0.0049, 0.29	0.057	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.77 (0.39) (54.24 - 58.22)	56.15 (0.40) (54.04 - 57.93)	-0.39 (0.16) (-1.42 - 0.80)	-0.72, -0.053	0.026	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.18 (0.022) (0.14 - 0.34)	0.17 (0.022) (0.12 - 0.30)	0.011 (0.0068) (-0.0073 - 0.052)	-0.0038, 0.025	0.136	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.29 (0.0086) (0.23 - 0.32)	0.28 (0.0087) (0.23 - 0.32)	0.0044 (0.0047) (-0.027 - 0.046)	-0.0057, 0.015	0.364	0.17, 0.38 (0.20 - 0.36)

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.15 (0.0051) (0.12 - 0.19)	0.15 (0.0051) (0.13 - 0.21)	-0.0035 (0.0029) (-0.049 - 0.032)	-0.0098, 0.0029	0.260	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0093) (0.10 - 0.22)	0.13 (0.0093) (0.081 - 0.19)	0.018 (0.0022) (-0.012 - 0.038)	0.013, 0.023	<0.001	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	8.90 (0.70) (5.22 - 11.91)	8.93 (0.70) (5.40 - 11.92)	-0.025 (0.16) (-2.59 - 1.29)	-0.34, 0.29	0.875	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	67.21 (4.40) (41.96 - 83.17)	71.33 (4.48) (45.03 - 95.10)	-4.12 (2.74) (-38.15 - 12.79)	-9.96, 1.71	0.153	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.40 (0.0083) (0.35 - 0.44)	0.38 (0.0084) (0.33 - 0.44)	0.021 (0.0032) (-0.036 - 0.054)	0.015, 0.028	<0.001	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	12.81 (0.47) (10.18 - 14.81)	11.73 (0.48) (8.61 - 14.11)	1.08 (0.28) (-1.95 - 2.54)	0.48, 1.68	0.001	9.07, 17.33 (9.07 - 17.14)

Table VI-2. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.72 (0.031) (0.56 - 0.84)	0.72 (0.031) (0.54 - 0.87)	0.0081 (0.0067) (-0.087 - 0.11)	-0.0053, 0.021	0.230	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.12 (0.028) (0.98 - 1.24)	1.07 (0.028) (0.79 - 1.27)	0.053 (0.020) (-0.12 - 0.27)	0.0089, 0.097	0.021	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.034 (0.0095) (0.018 - 0.12)	0.029 (0.0096) (0.0053 - 0.10)	0.0045 (0.0046) (-0.065 - 0.030)	-0.0053, 0.014	0.346	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	37.58 (2.01) (27.31 - 46.74)	40.14 (2.02) (28.22 - 52.95)	-2.57 (0.77) (-11.57 - 3.27)	-4.22, -0.91	0.005	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	140.14 (9.87) (86.23 - 179.34)	131.33 (9.88) (91.78 - 162.98)	8.80 (2.07) (-6.54 - 26.36)	4.39, 13.22	<0.001	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 was treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table VI-3. Statistical Summary of Combined-Site Cottonseed Anti-nutrients for MON 88701 vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0034) (0.11 - 0.19)	0.14 (0.0037) (0.11 - 0.17)	0.013 (0.0044) (-0.026 - 0.068)	0.0044, 0.022	0.003	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.39 (0.015) (0.20 - 0.55)	0.37 (0.016) (0.26 - 0.49)	0.013 (0.015) (-0.16 - 0.16)	-0.016, 0.043	0.371	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.22 (0.0067) (0.13 - 0.29)	0.21 (0.0072) (0.17 - 0.27)	0.0067 (0.0081) (-0.085 - 0.078)	-0.0096, 0.023	0.412	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.94 (0.037) (0.80 - 1.18)	0.89 (0.037) (0.68 - 1.20)	0.055 (0.020) (-0.086 - 0.20)	0.012, 0.099	0.016	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.04 (0.037) (0.84 - 1.24)	0.97 (0.037) (0.74 - 1.10)	0.066 (0.017) (-0.021 - 0.23)	0.031, 0.10	<0.001	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 was treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table VI-4. Literature and ILSI Ranges for Components in Cottonseed

Cottonseed Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Cottonseed Nutrients		
Proximates (% dw)		
Ash	3.87 – 5.29[a]; 3.7 – 4.2[d]	3.761 – 5.342
Carbohydrates by calculation	45.28 – 53.62[a]	39.0 – 53.6
Calories by calculation (Kcal/100g)	471.34 – 506.95[a]	Not available
Moisture (% fw)	2.25 – 7.49[a]	2.3 – 9.9
Protein	24.54 – 30.83[a]; 21.2 – 25.9[b]	21.48 – 32.97
Total Fat	17.37 – 25.16[a]; 14.4 – 16.9[d]	17.201 – 27.292
Fiber (% dw)		
Acid Detergent Fiber	21.10 – 34.8[a]; 37.6 – 40.5[d]	19.74 – 38.95
Neutral Detergent Fiber	32.92 – 45.83[a]; 50.0 – 53.6[d]	25.56 – 51.87
Crude Fiber	13.85 – 17.94[a]	13.86 – 23.10
Total Dietary Fiber	not available	33.69 – 47.55
Amino Acids	**(% total AA)**	**(% dw)**
Alanine	4.16 – 4.41[a]; 3.6 – 4.2[b]	0.80 – 1.22
Arginine	11.28 – 12.51[a]; 10.9 – 12.3[b]	2.06 – 3.72
Aspartic acid	9.73 – 9.99[a]; 8.8 – 9.5[b]	1.82 – 2.94
Cystine/Cysteine	1.60 – 1.92[a]; 2.3 – 3.4[b]	0.35 – 0.56
Glutamic acid	20.76 – 21.61[a]; 20.5 – 22.4[b]	3.91 – 6.72
Glycine	4.44 – 4.58[a]; 3.8 – 4.5[b]	0.83 – 1.32
Histidine	3.00 – 3.12[a]; 2.6 – 2.8[b]	0.57 – 0.91
Isoleucine	3.10 – 3.67[a]; 3.0 – 3.4[b]	0.62 – 1.05
Leucine	6.27 – 6.65[a]; 5.5 – 6.1[b]	1.14 – 1.86
Lysine	4.85 – 5.37[a]; 4.2 – 4.6[b]	0.94 – 1.46
Methionine	1.46 – 1.88[a]; 1.3 – 1.8[b]	0.30 – 0.47
Phenylalanine	5.56 – 5.77[a]; 5.0 – 5.6[b]	1.02 – 1.72
Proline	4.06 – 4.28[a]; 3.1 – 4.0[b]	0.75 – 1.23
Serine	4.45 – 4.86[a]; 3.9 – 4.4[b]	0.91 – 1.35
Threonine	3.26 – 3.59[a]; 2.8 – 3.2[b]	0.55 – 0.92
Tryptophan	0.97 – 1.21[a]; 1.0 – 1.4[b]	0.194 – 0.319
Tyrosine	2.65 – 2.92[a]; 2.8 – 3.3[b]	0.53 – 0.84
Valine	4.76 – 5.14[a]; 4.3 – 4.7[b]	0.87 – 1.49
Fatty Acids (% total FA)		
8:0 Caprylic	not available	not available
10:0 Capric	not available	not available
12:0 Lauric	not available	not available
14:0 Myristic	0.55 – 2.40[a]; 0.6 – 1.5[b]	0.455 – 2.400
14:1 Myristoleic	not available	not available
15:0 Pentadecanoic	0.050 – 0.17[a]	0.103 – 0.481
15:1 Pentadecenoic	not available	not available
16:0 Palmitic	21.23 – 27.9[a]; 17.6 – 24.8[b]	15.11 – 27.90
16:1 Palmitoleic	0.55 – 1.16[a]	0.464 – 1.190
17:0 Heptadecanoic	not available	0.092 – 0.119

Table VI-4. Literature and ILSI Ranges for Components in Cottonseed (continued)

Cottonseed Tissue Components[1]	Literature Range[2]	ILSI Range[3]
17:1 Heptadecenoic	not available	not available
18:0 Stearic	1.99 – 3.11 [a]; 2.0 – 2.5 [b]	0.20 – 3.11
18:1 Oleic	13.90 – 20.10 [a]; 15.0 – 20.7 [b]	12.8 – 25.3
18:2 Linoleic	46.00 – 56.88 [a]	46.0 – 59.4
18:3 Gamma Linolenic	0.050 – 0.25 [a]	0.097 – 0.232
18:3 Linolenic	0.050 – 0.25 [a]	0.11 – 0.35
20:0 Arachidic	0.25 – 0.33 [a]	0.186 – 0.414
20:1 Eicosenoic	not available	0.095 – 0.098
20:2 Eicosadienoic	not available	not available
20:3 Eicosatrienoic	not available	not available
20:4 Arachidonic	not available	not available
22:0 Behenic	0.13 – 0.17 [a]	0.104 – 0.295
Vitamins	**(mg/kg fw)**	**(mg/kg dw)**
Vitamin E	99 – 224[c]	70.825 – 197.243
Minerals (% dw)		
Calcium	0.10 – 0.33[a]	0.10323 – 0.32581
Copper (mg/kg dw)	3.54 – 11.14[a]	3.13 – 24.57
Iron (mg/kg dw)	40.58 – 56.54[a]	36.71 – 318.38
Magnesium	0.37 – 0.46[a]	0.34709 – 0.49312
Manganese (mg/kg dw)	11.06 – 18.31[a]	10.69 – 21.96
Phosphorus	0.60 – 0.84[a]	0.48254 – 0.99157
Potassium	0.98 – 1.24[a]	0.98345 – 1.44835
Sodium	0.0054 – 0.74[a]	0.01118 – 0.73548
Zinc (mg/kg dw)	30.21 – 47.75[a]	27.0 – 59.5
Cottonseed Anti-Nutrients		
Gossypol, Total (% dw)	0.57 – 1.42[a]; 0.55 – 0.77[d]	0.547 – 1.522
Gossypol, Free (% dw)	0.53 – 1.20[a]	0.454 – 1.399
Cyclopropenoid Fatty Acids (% total FA)		
Dihydrosterculic	0.13 – 0.24 [a]	0.075 – 0.310
Malvalic	0.33 – 0.58 [a]	0.229 – 0.759
Sterculic	0.21 – 0.56 [a]	0.190 – 0.556

[1] fw=fresh weight; dw=dry weight
[2] Literature range references; [a](Hamilton et al., 2004); [b](Lawhon et al., 1977); [c](Smith and Creelman, 2001); [d](Bertrand et al., 2005).
[3] (ILSI, 2011).

VI.B. Compositional Assessment of MON 88701 Conclusion

Detailed analyses were conducted on nutrient and anti-nutrient levels in MON 88701 cottonseed from plants treated with dicamba and glufosinate, reported above, and plants not treated with dicamba or glufosinate (Appendix E). Component levels for MON 88701 were compared to levels in the conventional control. The analytes evaluated are consistent with those identified by the OECD as important to understanding the safety and nutrition of new varieties of biotechnology-derived cotton (OECD, 2009). These compositional comparisons were made by analyzing the acid-delinted cottonseed harvested from plants grown at each of eight field sites in the U.S. during the 2010 field season. Composition analyses of all samples, conducted in accordance with OECD guidelines, were performed for nutrients including proximates (ash, carbohydrates, and calories by calculation, moisture, protein, and fat), fibers (ADF, CF, NDF, and TDF), amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamin E. The anti-nutrients assessed in this analysis included total and free gossypol and cyclopropenoid fatty acids (dihydrosterculic, malvalic, and sterculic). These analyses also included measurements of the same nutrients and anti-nutrients in conventional commercial cotton varieties, known as reference varieties, to provide data on natural variability of each compositional component analyzed. All cotton plants including MON 88701, the conventional control, and the conventional commercial reference varieties were treated with maintenance pesticides as necessary throughout the growing season. In addition, MON 88701 plots were treated at the 3-5 leaf stage with glufosinate herbicide at the label rate (0.5 lbs a.i./acre) and at the 6-10 leaf stage with dicamba herbicide at the label rate (0.5 lbs a.e./acre).

For MON 88701 compared to the conventional control, the combined-site analysis of cottonseed showed no statistically significant differences ($p<0.05$) between nutrient and anti-nutrient components of MON 88701 and the control for 30 (57.7%) of the 52 mean value comparisons. Cottonseed nutrient component differences included mean values for five proximates (ash, calories, carbohydrates, moisture, and total fat), three types of fiber (ADF, NDF, and TDF), three amino acids (arginine, methionine, and proline), two fatty acids (14:0 myristic acid and 18:2 linoleic acid), five minerals (calcium, magnesium, manganese, potassium and zinc), and vitamin E. Cottonseed anti-nutrient component differences included mean values for dihydrosterculic acid, free and total gossypol. All nutrient and anti-nutrient component differences observed in the combined-site statistical analysis, whether reflecting increased or decreased MON 88701 mean values with respect to the conventional control, were 14.09% or less. Mean values for all significantly different nutrient and anti-nutrient components from the combined-site analysis of MON 88701, with the exception of methionine, were within the 99% tolerance interval established from the conventional, commercial reference varieties grown concurrently in the same trial. All combined-site mean values, including methionine, and individual site mean values of MON 88701 for all nutrient and anti-nutrient components were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Overall, for MON 88701 mean component values observed to be significantly different from those of the conventional control, the differences with the control were generally shown to be of small relative magnitudes. All MON 88701 mean component values in the combined-site analysis, with the exception of methionine, were within the 99% tolerance interval established from the conventional commercial references varieties grown concurrently and at the same field sites. All combined-site mean values including methionine and individual site mean values of MON 88701 for all nutrient and anti-nutrient components were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

For MON 88701 treated with dicamba and glufosinate, compared to the conventional control, most of the combined-site differences were not reproducible among the individual sites, with the exception of ash and calcium; however, all of the combined-site component values were within the range of values reported in the scientific literature and/or in the ILSI Crop Composition Database. Additionally, the concentrations of key nutrients and anti-nutrients of cottonseed from MON 88701 that was not treated with dicamba or glufosinate were also analyzed (See Appendix E). Results from this analysis were similar to those of the dicamba and glufosinate treated analysis. Based on the results of this composition analysis, it is concluded that cottonseed from MON 88701 is compositionally equivalent to conventional cotton and therefore the food and feed safety and nutritional quality of this product is comparable to that of the commercially cultivated cotton.

Conventional cotton processing is described in Section II of this document. The processing of MON 88701 is not expected to be any different from that of conventional cotton. As described in this section, detailed compositional analyses of key components of MON 88701 have been performed and have demonstrated that MON 88701 is compositionally equivalent to conventional cotton. Additionally, the mode of action of the MON 88701 DMO and PAT (*bar*) proteins, as described in Section V.A., is well understood, and there is no reason to expect interactions with important nutrients or known anti-nutrients that are present in cotton. Therefore, when MON 88701 and its progeny are used on a commercial scale as a source of food or feed, these products are not expected to be different from the equivalent foods or feeds originating from commercially cultivated cotton.

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides a comparative assessment of the phenotypic, agronomic, and environmental interaction characteristics of MON 88701 compared to the conventional control. The data support a conclusion that MON 88701 is not meaningfully different from the conventional control with the exception of the dicamba and glufosinate-tolerance traits, and therefore is no more likely to pose a plant pest risk or have a significant environmental impact compared to conventional cotton. These conclusions are based on the results of multiple evaluations from laboratory and field experiments.

Phenotypic, agronomic, and environmental interaction characteristics of MON 88701 were evaluated in a comparative manner to assess plant pest potential. These assessments included evaluation of seed germination characteristics, plant growth and development characteristics, pollen characteristics, observations of plant responses to abiotic stress, and plant-disease and plant-arthropod interactions. Results from these assessments demonstrate that MON 88701 does not possess: a) increased weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stressors, diseases, or arthropods; or c) characteristics that would confer a plant pest risk or a significant environmental impact compared to the conventional control.

VII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 88701, data were collected to evaluate altered plant pest potential. A detailed description of the regulated article phenotype is requested as part of the petition for determination of nonregulated status in 7 CFR § 340.6 including differences from the unmodified recipient organism that would "substantiate that the regulated article is unlikely to pose a greater plant pest risk than unmodified organism from which it was derived." As part of the characterization of MON 88701, data were collected to provide a detailed description of the phenotypic, agronomic, and environmental interactions characteristics of MON 88701.

The plant characterization of MON 88701 encompassed five general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) plant mapping; 5) plant response to abiotic stress and interactions with diseases and arthropods. An overview of the characteristics assessed is presented in Table VII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity (OECD, 1993) and were comprised of a combination of field and laboratory studies conducted by scientists who are familiar with the production and evaluation of cotton. In each of these assessments, MON 88701 was compared to a conventional control, Coker 130, which has a genetic background similar to MON 88701, but does not possess the dicamba and glufosinate-tolerance traits. In addition, multiple commercial reference varieties developed through conventional selection and breeding (See Appendices F-H and Tables F-1, G-1, G-2, and H-1) were included to provide a

range of comparative values that are representative of the variability in existing commercial cotton varieties for each characteristic. Data collected for the various characteristics from the commercial reference varieties provides context for interpreting experimental results.

Table VII-1. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated in United States Field Trials and Laboratory Studies

Data Category	Characteristics measured (associated section where discussed)[1]	Evaluation timing (setting of evaluation)[2]	Evaluation description (measurement endpoints)
Seed germination, dormancy, and emergence	Normal germinated (VII.C.1.)	Day 4 and 12 (20/30ºC) (laboratory)	Percentage of seed producing seedlings exhibiting normal developmental characteristics
	Abnormal germinated (VII.C.1.)	Day 12 (20/30°C) (laboratory)	Percentage of seed producing seedlings that could not be classified as normal germinated
	Germinated (VII.C.1.)	Day 4, 12, and 18 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that had germinated normally and abnormally
	Dead (VII.C.1.)	Day 4 and 12 (10, 20, 30, 10/20, 10/30, and 20/30°C); Day 18 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that had visibly deteriorated and become soft to the touch (also included non-viable hard and nonviable firm-swollen seed)
	Viable hard (VII.C.1.)	Day 12 (20/30°C); Day 18 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that did not imbibe water and remained hard to the touch (viability determined by a tetrazolium test[2])
	Viable firm-swollen (VII.C.1.)	Day 12 (20/30°C); Day 18 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that imbibed water and were firm to the touch but did not germinate (viability determined by a tetrazolium test[3])
	Stand count (VII.C.2.1.)	Approximately 14 and 30 DAP (Field)	Number of emerged plants in two rows, standardized to 20 ft rows
	Final stand count (VII.C.2.1.)	Within approximately 7 days of harvest (Field)	Number of plants in two rows, standardized to 20 ft rows
Vegetative Growth	Plant vigor (VII.C.2.1.)	Approximately 14 and 30 DAP (Field)	Rated on a 1-9 scale, where 1 = excellent, 5 = average, and 9 = poor vigor
	Plant height (cm) (VII.C.2.1.)	Approximately 30 DAP and within approximately 7 days of harvest (Field)	Distance from cotyledonary node (0 node) to the uppermost terminal bud on 10 plants from two rows
	Nodes above white flower (NAWF) (VII.C.2.1.)	Three weekly observations starting approximately 7 days after first flower (Field)	Number of nodes from upper most first-position white flower to the terminal bud on 10 plants from two rows

Table VII-1. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated in United States Field Trials and Laboratory Studies (continued)

Data Category	Characteristics Measured (associated section where discussed)	Evaluation timing (setting of evaluation)	Evaluation description (measurement endpoints)
Reproductive Development	Seedcotton yield (kg/ha) (VII.C.2.1.)	At harvest (Field)	Hand harvested all seedcotton from two rows
	Seed index (g per 100 seed) (VII.C.2.1.)	Post harvest (Field)	Mass of 100 ginned, fuzzy seed
	Total seed per boll (VII.C.2.1.)	Post harvest (Field)	Average number of seeds per boll calculated from a 50-boll sample
	Mature seed per boll (VII.C.2.1.)	Post harvest (Field)	Average number of mature seed in a boll calculated from a 50-boll sample
	Immature seed per boll (VII.C.2.1.)	Post harvest (Field)	Average number of immature seed in a boll calculated from a 50-boll sample
	Boll weight (g) (VII.C.2.1.)	Post harvest (Field)	Average mass of a single boll calculated from a 50-boll sample
	Fiber micronaire (mic units) (VII.C.2.1.)	Post harvest (Field)	Measure of fiber fineness and maturity (expressed in dimensionless micronaire (mic) units)
	Fiber elongation (%) (VII.C.2.1.)	Post harvest (Field)	Measure of the tensile-elastic behavior of the fiber. It is a measure of how much the fibers stretch before they tear.
	Fiber strength (g/tex) (VII.C.2.1.)	Post harvest (Field)	Force in grams required to break a bundle of fibers one tex unit in size. One tex is the mass in grams of 1,000 meters of fiber.
	Fiber length (cm) (VII.C.2.1.)	Post harvest (Field)	Average length of the longer half of combed fibers
	Fiber uniformity (%) (VII.C.2.1.)	Post harvest (Field)	Ratio between the mean length and the longer half mean length of fibers
	Pollen viability (VII.C.3.)	Flowering (laboratory)	Percentage of viable pollen; viable pollen stains purple due to the presence of vital cytoplasmic content
	Pollen morphology (VII.C.3.)	Flowering (laboratory)	Diameter (µm) of viable pollen grains and observations

Table VII-1. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated in United States Field Trials and Laboratory Studies (continued)

Data Category	Characteristics measured (associated section where discussed)	Evaluation timing (setting of evaluation)	Evaluation description (measurement endpoints)
Plant Mapping Characteristics	Number of mainstem nodes per plant (VII.C.2.2.)	At harvest (Field)	Number of mainstem nodes from cotyledonary node (node 0) to uppermost terminal meristem on 10 plants per plot
	Number of nodes to first fruiting branch per plant (VII.C.2.2.)	At harvest (Field)	Number of nodes from cotyledonary node (node 0) up to first fruiting branch on 10 plants per plot
	Number of first-position bolls (total, normal & abnormal) per plant (VII.C.2.2.)	At harvest (Field)	Number of bolls at first position on fruiting branches off of mainstem on 10 plants per plot
	Number of vegetative bolls per plant (VII.C.2.2.)	At harvest (Field)	Number of vegetative bolls on 10 plants per plot
	Total bolls per plant (VII.C.2.2.)	Post harvest (Field)	Sum of first-position bolls, second-position bolls, and vegetative bolls per plant on 10 plants per plot
	Retention of first-position bolls (%) (VII.C.2.2.)	Post harvest (Field)	Calculated first-position bolls relative to number of fruiting branches on the mainstem
	First-position bolls (%) (VII.C.2.2.)	Post harvest (Field)	Calculated first-position bolls per plant relative to total bolls per plant

Table VII-1. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated in United States Field Trials and Laboratory Studies (continued)

Data Category	Characteristics measured (associated section where discussed)	Evaluation timing (setting of evaluation)	Evaluation description (measurement endpoints)
Plant-environmental interactions	Plant response to abiotic stress (VII.C.2.3.)	Four times per growing season – approximately 30, 60, 90 and 120 DAP (Field)	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Disease damage (VII.C.2.3.)	Four times per growing season – approximately 30, 60, 90 and 120 DAP (Field)	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Arthropod-related damage (VII.C.2.3.)	Four times per growing season – approximately 30, 60, 90 and 120 DAP (Field)	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Thrips damage assessment (VII.C.2.3.)	Three assessments at approximately 14, 21 and 28 DAP (Field)	Specific quantitative assessment of thrips from 10 plants in each plot using a 0-5 scale, where 0 = no thrips or visible damage and 5 = numerous thrips or severe damage from thrips
	Heliothine damage assessment (VII.C.2.3.)	Four assessments at approximately 45, 60, 75 and 90 DAP (Field)	Percent damage (number of damaged fruiting bodies divided by total number of fruiting bodies) and number live larvae on the top 7 nodes of 10 plants in each plot
	Arthropod abundance (VII.C.2.3.)	Four collections at approximately 30, 60, 90 and 120 DAP (Field)	Number of pest and beneficial arthropods

[1]All cottonseed was from mature open bolls.
[2]Cotton plant growth stages were determined using descriptions and guidelines outlined in Cotton Growth and Development ([redacted] et al., 2007).
[3]Viability of hard and firm-swollen seed were determined by a tetrazolium test (AOSA, 2007).

VII.B. Interpretation of Phenotypic and Environmental Interaction Data

Plant pest risk assessments for biotechnology-derived crops are comparative assessments. Familiarity provides a basis from which the potential environmental impact of a biotechnology-derived plant can be evaluated. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a well-characterized conventional plant variety. Familiarity considers the biology of the crop, the introduced trait(s), 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 cotton was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for cotton. As such, MON 88701 was compared to the conventional control, Coker 130, in the assessment of measured characteristics. An overview of the characteristics assessed is presented in Table-VII-1. Evaluation of environmental interaction characteristics (*e.g.*, plant abiotic stress, plant-disease, and plant-arthropod interactions) was also considered in the plant pest assessment. Based on all of the data collected, an assessment was made to determine if MON 88701 is likely to pose an increased plant pest risk compared to commercial cotton. Prior to analysis, the overall dataset was evaluated for possible evidence of biologically relevant changes and an unexpected plant response. No unexpected observations or issues were identified.

VII.B.1. Interpretation of Detected Differences Criteria

Comparative plant characterization data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest/weed 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/weed potential of the biotechnology-derived crop compared to the conventional crop. Characteristics for which differences are detected are considered in a step-wise method (Figure VII-1) or in a similar fashion. All detected differences for a characteristic are considered in the context of whether or not the difference would increase the plant pest/weed potential of the biotechnology-derived crop. Ultimately, a weight-of-evidence approach considering all characteristics and data is used for the overall risk assessment of differences and their significance. In detail, Figure VII-1 illustrates the stepwise assessment process employed:

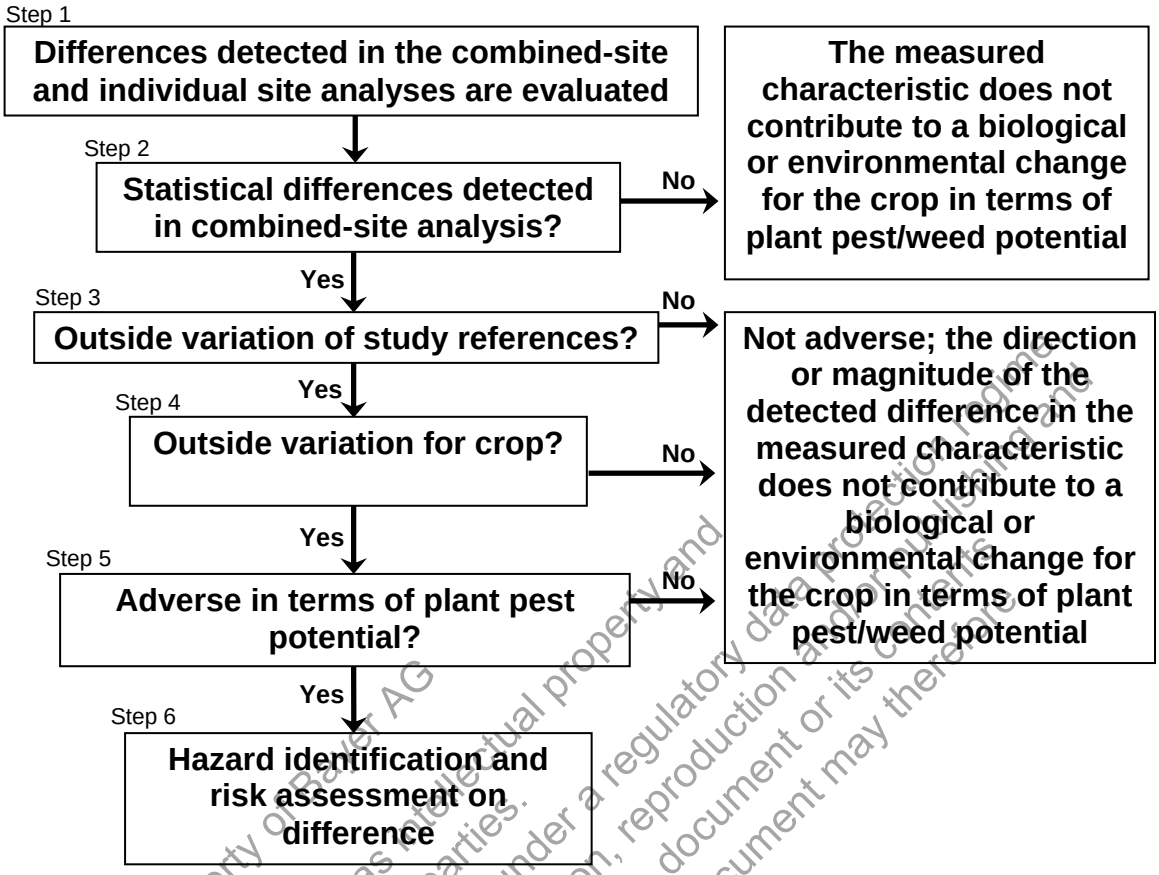


Figure VII-1. Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods

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

Steps 1 and 2 - Evaluate Detected Statistically Significant Differences

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

Step 3 - Evaluate differences in the context of commercial reference varieties included in the Study

If a difference for a characteristic is detected in the combined-site analysis across multiple environments, then the mean value of the biotechnology-derived crop for the

characteristic is assessed relative to the range of variation of the commercial reference varieties included in the study (e.g., reference range).

Step 4 - Evaluate Differences in the Context of the Crop

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

Step 5 - Relevance of Difference to Plant Pest/Weed Potential

If the mean value of the characteristics for a biotechnology-derived crop is outside the range of values common for the crop, the detected difference for the characteristic is then assessed for whether or not it is adverse in terms of plant pest/weed 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/weed potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for and effects of trait introgression into any populations growing outside of cultivated environments or into a sexually-compatible species.

VII.B.1.1. Interpretation of Vigor and Environmental Interactions Data

For the qualitative assessments of vigor and abiotic stress response, disease damage, and arthropod damage, the biotechnology-derived crop and conventional control were considered different in plant response ratings if the range of values or injury symptoms did not overlap between the biotechnology-derived crop and the conventional control across all four replications. Any observed differences between the biotechnology-derived crop and conventional control were assessed for biological significance in the context of the range of the commercial reference varieties, and consistency in other observation times and sites. Differences that are not consistently observed at other observations/collections and sites are considered not biologically meaningful in terms of plant pest potential or an adverse environmental impact.

Quantitative assessments of arthropod damage were analyzed within individual sites and pooled across sites in a combined-site analysis. Statistically significant differences detected between the biotechnology-derived crop and conventional control were evaluated using the method outlined in Figure VII-1.

Quantitative assessments of arthropod abundance were only analyzed within each individual site. Statistically significant differences between the biotechnology-derived crop and conventional control were assessed for biological significance in the context of the range of the commercial reference varieties, and for consistency with other collection times and collection sites. Differences that are not consistently detected at other times

and sites are considered not biologically meaningful in terms of plant pest potential or an adverse environmental impact.

VII.C. Comparative Assessments of the Phenotypic, Agronomic, Plant Mapping, and Environmental Interaction Characteristics of MON 88701

This section provides the results of comparative assessments conducted in replicated laboratory and multi-site field experiments to provide a detailed phenotypic, agronomic, plant mapping, and environmental interaction description of MON 88701. The MON 88701 characteristics evaluated in these assessments included: seed dormancy and germination characteristics (Section VII.C.1.), plant phenotypic, plant mapping, and environmental interaction observations under field conditions (Section VII.C.2.), and pollen characteristics (Section VII.C.3). Additional details for each assessment are provided in Appendices F through H.

VII.C.1. Seed Dormancy and Germination Characteristics

USDA-APHIS considers the potential for weediness to constitute a plant pest factor (7 CFR § 340.6). Seed germination and dormancy mechanisms vary among species and their genetic basis tends to be complex. Seed dormancy (*e.g.*, hard seed) is an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996; [redacted] and [redacted], 2007). Cotton does not exhibit significant levels of seed dormancy as this characteristic has been removed through selection and conventional breeding (Christiansen and Moore, 1959). To assess germination characteristics, standardized germination assays are routinely used. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends a temperature range of alternating 20/30°C as optimal for testing the germination characteristics of cottonseed (AOSA, 2007; 2010a; 2010b; AOSA/SCST, 2010).

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 88701 and the conventional control. In addition, nine unique commercial reference varieties were included to provide a range of comparative values that are representative of existing variability in commercial cotton varieties. The seed lots for MON 88701, the conventional control, and the commercial reference varieties were produced in three replicated field trials during 2010 located in Arkansas (ARPR), North Carolina (NCME), and Texas (TXPL). These geographic areas represent environmentally relevant conditions for cotton production. In addition to the AOSA recommended temperature range of 20/30°C, seed was tested at five additional temperature regimes of 10, 20, 30, alternating 10/20, and alternating 10/30°C to assess seed germination properties. The details of the materials, experimental methods, and germination data from all of the individual production sites are presented in Appendix F.

In the combined-site analysis, in which the data were pooled from the three individual sites, no statistically significant differences (5% level of significance) were detected between MON 88701 and the conventional control for any characteristic at the AOSA temperature regime (alternating $20^{o}C/30^{o}C$), or at the temperature regimes of $10^{o}C$, $20^{o}C$, alternating $10^{o}C/20^{o}C$, or alternating $10^{o}C/30^{o}C$ (Table VII-2). MON 88701 had a

significantly higher percentage of germinated seed (96.7 vs. 94.4, respectively) and lower percent dead seed (3.3 vs. 5.6, respectively) than the conventional control at 30°C. These differences were small in magnitude, not observed at other temperatures and the mean values of percent germinated and dead seed for MON 88701 were within the range of commercial reference varieties. Therefore, the differences in percent germinated and dead seed at 30°C are not considered to be biologically meaningful in terms of altered dormancy or germination characteristics (See Figure VII- 1 Step 3, answer "no").

The dormancy and germination characteristics evaluated were used to assess MON 88701 in the context of plant pest risk. The results of this assessment, particularly the fact that no hard seed were observed at any temperature, support the conclusion that there are no seed germination characteristic differences between MON 88701 and the conventional control. Thus, the introduction of the dicamba and glufosinate-tolerant traits into cotton is not likely to result in increased plant pest potential, increased weediness, or an altered environmental impact from MON 88701 compared to commercially cultivated cotton.

Table VII-2. Combined-Site Comparison of MON 88701 to Conventional Control for Germination Characteristics

Temperature Regime	Germination Category	Mean % (S.E.)[1]		
		MON 88701[2]	Control	Reference Range (%)[3]
10 °C	Germinated	32.8 (6.3)	34.8 (4.7)	14.2 - 58.0
	Viable Hard	0.0 (0.0)	0.3 (0.2)	0.2 - 23.8
	Dead	22.8 (3.8)	22.1 (3.3)	7.7 - 22.8
	Viable Firm-Swollen	44.3 (7.3)	42.8 (5.9)	29.0 - 66.5
20 °C	Germinated	95.7 (0.7)	95.3 (1.2)	88.5 - 97.8
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 - 4.0
	Dead	4.3 (0.7)	4.7 (1.2)	2.0 - 9.0
	Viable Firm-Swollen	0.0 (0.0)	0.0 (0.0)	0.0 - 2.0
30 °C	Germinated	96.7 (0.8)*	94.4 (1.2)	90.5 - 97.8
	Viable Hard†	0.0 (0.0)	0.0 (0.0)	0.0 - 0.0
	Dead	3.3 (0.8)*	5.6 (1.2)	2.3 - 9.5
	Viable Firm-Swollen†	0.0 (0.0)	0.0 (0.0)	0.0 - 0.0
10/20 °C	Germinated	94.4 (1.3)	92.0 (1.4)	64.3 - 91.3
	Viable Hard	0.0 (0.0)	0.1 (0.1)	0.1 - 17.5
	Dead	4.3 (0.7)	6.6 (1.2)	5.0 - 10.3
	Viable Firm-Swollen	1.3 (1.0)	1.3 (0.6)	0.0 - 21.3
10/30 °C	Germinated	95.7 (1.0)	95.1 (1.5)	90.8 - 95.5
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 - 2.0
	Dead	4.3 (1.0)	4.9 (1.5)	4.3 - 7.8
	Viable Firm-Swollen	0.0 (0.0)	0.0 (0.0)	0.0 - 1.5
20/30 °C (AOSA)[4]	Normal Germinated	89.6 (1.9)	88.0 (2.9)	80.8 - 92.8
	Abnormal Germinated	4.8 (1.2)	6.0 (1.3)	2.0 - 6.3
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 - 4.8
	Dead	5.6 (0.9)	6.0 (2.0)	4.0 - 10.8
	Viable Firm-Swollen	0.1 (0.1)	0.0 (0.0)	0.0 - 3.8

Note: The experimental design was a split-plot with four replications (n = 12) and statistical analysis consisted of an analysis of variance (ANOVA) model.

*Statistically significant differences detected (α=0.05) between MON 88701 and the conventional control.

[1]S.E. = Standard Error.

[2]In some instances, the total percentage of MON 88701 did not equal 100% due to numerical rounding of the means.

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

[4]AOSA recommended.

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

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

Phenotypic, agronomic, and plant mapping characteristics, and environmental interactions were evaluated under field conditions as part of the plant characterization assessment of MON 88701. These data were developed to provide USDA-APHIS with a detailed description of MON 88701 relative to the conventional control and commercial reference varieties. According to 7 CFR § 340.6, as part of the petition to seek deregulation, a petitioner must submit "a detailed description of the phenotype of the regulated article." This information is being provided to assess whether there are phenotypic differences between MON 88701 and the conventional control that may impact its plant pest/weed potential. Environmental interactions were also assessed as an indirect indicator of phenotypic changes to MON 88701 compared to the same comparators described above and are also considered in the plant pest assessment.

The results of the assessment of agronomic, phenotypic, and plant mapping characteristics demonstrated that the introduction of the dicamba and glufosinate-tolerance traits did not meaningfully alter the weediness of MON 88701 compared to the conventional control. Furthermore, the lack of meaningful differences in plant response to abiotic stress, disease damage, arthropod-related damage, and pest- and beneficial-arthropod abundance also support the conclusion that the introduction of the dicamba and glufosinate-tolerance traits are not likely to result in increased plant pest potential, increased weediness, or an adverse environmental impact from MON 88701 compared to the conventional control.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

Two field studies were conducted during 2010 to evaluate phenotypic and agronomic characteristics of MON 88701 compared to the conventional control. One study was designed to collect phenotypic and environmental interaction data, while the other was designed for the collection of plant mapping data and tissue samples for expression and compositional analyses. Field sites in both studies were planted in randomized complete block designs with four replicates per site. The sites were selected to provide a diverse range of environmental and agronomic conditions representative of commercial cotton production areas in North America (Table VII-3). All plots of MON 88701, the conventional control, and the commercial reference varieties at each site were uniformly managed in order to assess whether the introduction of the dicamba and glufosinate-tolerance traits altered the phenotypic and agronomic characteristics of MON 88701 compared to the conventional control. Both studies included MON 88701 that was not treated with dicamba or glufosinate herbicides to assess the effects of the traits on the plant.

Study 1 was conducted at 15 sites in the U.S. (Table VII-3). MON 88701, the conventional control, and four commercial reference varieties (three conventional reference varieties and one glyphosate-tolerant reference variety) were evaluated at each

site. Across sites, a total of 11 commercial reference varieties (Table G-1) were evaluated.

An additional study, Study 2, was conducted at 11 sites in the U.S. (Table VII-3). MON 88701, the conventional control, and four conventional reference varieties were evaluated at each site. Across sites a total of eight unique commercial reference varieties were evaluated. This study was designed for collection of plant mapping data, as well as, the production of tissues for the expression and compositional analyses discussed above in Sections V.C. and VI, respectively. Study 2 generated plant mapping information and data across test locations treated and not treated with dicamba or glufosinate herbicides in Study 2 (See section VII.C.2.2, C.2.3.2 and G.12.3), allowing for assessment of MON 88701 under the agronomic system that it is expected to be used.

Results from Study 1 and Study 2 are presented in the following sections:

Table VII-3. Study 1 and Study 2 Data Location Summary

Data/Results[1]	Study 1	Study 2	Petition Location
Not treated phenotypic characteristics	✓	✓	Section VII.C.2.1
Not treated plant mapping		✓	Section VII.C.2.2
Not treated environmental interactions	✓	✓	Section VII.C.2.3
Treated phenotypic characteristics		✓	Appendix G.12.3
Treated plant mapping		✓	Appendix G.13.2
Treated environmental characteristics through plant mapping		✓	Section VII.C.2.3; Appendix G.13.2

[1]Not treated = not treated with dicamba or glufosinate herbicides; treated = treated with dicamba and glufosinate herbicides.

All plant, seed, and fiber characteristic data, except for plant vigor (qualitative data), were statistically analyzed within each site (*i.e.*, individual site analysis) and in a combined-site analysis in which the data were pooled across all sites within a study. The reference range was determined from the minimum and maximum mean values from the commercial reference varieties to provide phenotypic characteristic values representative of commercial cotton varieties.

For the assessment of plant vigor MON 88701 and the conventional control were considered different in plant response rating if the range of values did not overlap between the MON 88701 and the conventional control across all four plot replications. Any observed differences between MON 88701 and the conventional control were assessed for biological significance in the context of the range of the commercial reference varieties, and for consistency in other observations and sites. Differences that

are not consistently observed at other observations and sites are considered not biologically meaningful in terms of plant pest potential or an adverse environmental impact.

A description of the evaluated phenotypic characteristics and the designated developmental stages when evaluations occurred are listed in Table VII-1. The results from Study 1 and 2 from combined-site analyses of MON 88701 plots not treated with either dicamba or glufosinate compared to the conventional control are presented in the following sub-sections. The results of these studies demonstrate that the introduction of the dicamba and glufosinate-tolerance traits did not alter MON 88701 compared to the conventional control in terms of weediness. The individual site data comparisons and methods and detailed results of the supplemental evaluations of MON 88701 plots that were treated with dicamba and glufosinate in Study 2 are presented and discussed in Appendix G.

Table VII-4. Field Phenotypic Evaluation Sites for MON 88701 during 2010

Study 1 Locations[1]		Study 2 Locations[2]	
County, State	Site Code	County, State	Site Code
Jackson, AR	ARAU	Crittenden, AR	ARPR
Crittenden, AR	ARPR	Desha, AR	ARTI
Desha, AR	ARTI	Tift, GA	GACH
Tift, GA	GACH	Twiggs, GA	GAJE
Twiggs, GA	GAJE	Pawnee, KS	KSLA
Pawnee, KS	KSLA	Rapides, LA	LACH
Rapides, LA	LABU	Perquimans, NC	NCBD
Rapides, LA	LACH	Caswell, NC	NCME
Perquimans, NC	NCBD	Dona Ana, NM	NMLC
Caswell, NC	NCME	Barnwell, SC	SCEK
Dona Ana, NM	NMGA	Hale, TX	TXPL
Dona Ana, NM	NMLC		
Barnwell, SC	SCEK		
Hale, TX	TXPL		
San Patricio, TX	TXPO		

Note: Field trials at all sites were conducted under USDA Notification number 10-071-101n.
[1]MON 88701 was not treated with dicamba or glufosinate herbicides in Study 1.
[2]Study 2 included plots not treated with dicamba or glufosinate herbicides and plots treated with dicamba and glufosinate herbicides.

VII.C.2.1.1. Field Phenotypic and Agronomic Characteristics of MON 88701 – Study 1

Vigor ratings were collected from each plot using a 1-9 scale, where 1 is outstanding plant vigor and 9 is poorest plant vigor. Since vigor data are categorical (qualitative), the data were not statistically analyzed. There were no differences observed between MON 88701 and the conventional control in plant vigor (Table G-7) at 14 and 30 days after planting (DAP) for 29 of 30 comparisons from all sites. At ARPR at 30 days after planting, MON 88701 had lower plant vigor than the conventional control (ranges of 4.0-4.0 vs. 2.0-3.0, respectively), but was within the range of the commercial reference varieties. Since only one difference (out of 30) was identified and it fell within the reference range, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Section VII.B.1.1.).

In the combined-site analysis (Table VII-5), no statistically significant differences were detected between MON 88701 and the conventional control for stand count at 14 and 30 DAP, final stand count, number of nodes above white flower at observation 1, seedcotton yield, number of immature seed per boll, weight per boll, fiber micronaire, fiber elongation, fiber uniformity, and fiber length. The following statistically significant differences were detected in the combined-site analysis. MON 88701 plants were shorter at 30 DAP (18.3 vs. 19.7 cm) and at harvest (109.8 vs. 116.4 cm), had increased nodes above white flower at observation 2 (6.0 vs. 5.7) and observation 3 (4.9 vs. 4.6), a decreased seed index (9.8 vs. 10.5 g per 100 fuzzy seed), increased total seed per boll (29.0 vs. 27.4), increased mature seeds per boll (22.6 vs. 19.7), and increased fiber strength (31.8 vs. 31.0 g/tex) compared to the conventional control. However, the mean values of MON 88701 were within the range of the commercial reference varieties for the eight characteristics listed above. Thus, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control. (See Figure VII-1, Step 3, answer "no").

Table VII-5. Study 1 Combined-Site Comparison of MON 88701 to Conventional Control during 2010 for Phenotypic and Agronomic Characteristics

		MON 88701	Control	Reference Range[1]	
Phenotypic Characteristic (units)		Mean (SE)[2]	Mean (SE)	Minimum	Maximum
Stand Count at 14 DAP[3]	(# in 2 rows per plot)	146.0 (4.3)	152.4 (4.2)	96.2	143.5
Stand Count at 30 DAP	(# in 2 rows per plot)	131.8 (5.5)	137.7 (5.5)	86.7	140.8
Final Stand Count at harvest	(# in 2 rows per plot)	125.2 (5.9)	128.9 (6.0)	88.2	131.4
Plant Height at 30 DAP (cm)		18.3 (1.2)*	19.7 (1.2)	8.3	23.3
Plant Height at harvest (cm)		109.8 (3.8)*	116.4 (4.2)	84.4	131.3
Nodes Above White Flower:	(# of nodes at observation 1)	6.9 (0.2)	6.7 (0.2)	5.8	8.6
	(# of nodes at observation 2)	6.0 (0.2)*	5.7 (0.2)	5.1	6.9
	(# of nodes at observation 3)	4.9 (0.3)*	4.6 (0.3)	3.7	5.7
Seedcotton Yield (kg/ha)		2937.8 (153.7)	2869.9 (156.0)	2107.0	3636.5
Seed Index (g per 100 fuzzy seed)		9.8 (0.2)*	10.5 (0.1)	8.9	11.8
Total Seed per Boll (# per boll)		29.0 (0.4)*	27.4 (0.3)	26.4	30.6
Mature Seed per Boll (# per boll)		22.6 (0.7)*	19.7 (0.6)	11.8	27.2
Immature Seed per Boll (# per boll)		6.4 (0.5)	7.7 (0.5)	3.4	16.0
Weight per Boll (g)		4.8 (0.1)	4.8 (0.1)	4.2	6.0
Fiber Micronaire (mic units)[4]		4.6 (0.1)	4.5 (0.1)	4.0	5.0
Fiber Elongation (%)		6.0 (0.1)	6.0 (0.1)	4.8	8.0
Fiber Strength (g/tex)		31.8 (0.2)*	31.0 (0.1)	30.7	34.5
Fiber Uniformity (%)		84.0 (0.1)	83.7 (0.1)	83.7	84.8
Fiber Length (cm)		2.8 (0.0)	2.9 (0.0)	2.8	3.1

* Indicates a statistically significant difference (α=0.05) between MON 88701 and the conventional control (n = 60).
[1] Reference range = Minimum and maximum mean values across all 15 sites and eleven references from the Study 1 field trial.
[2] SE = standard error.
[3] DAP = days after planting.
[4] Measure of fiber fineness and maturity (expressed in dimensionless micronaire units).

VII.C.2.1.2. Field Phenotypic and Agronomic Characteristics of MON 88701 – Study 2

Vigor ratings were collected from each plot using a 1-9 scale, where 1 is outstanding plant vigor and 9 is poorest plant vigor. Due to the non-specific nature of the scale used, the data were not statistically analyzed. There were no differences between MON 88701 and the conventional control in plant vigor (Table G-10) at 14 and 30 DAP for 22 of 22 comparisons from all sites. Therefore, the lack of differences in plant vigor supports a conclusion that MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Section VII.B.1.1.).

In the combined-site analysis of MON 88701 (Table VII-6), no statistically significant differences were detected between MON 88701 and the conventional control for stand count at 14 and 30 DAP, final stand count at harvest, nodes above white flower observations 1 and 3, seedcotton yield, immature seed per boll, weight per boll, fiber micronaire, fiber elongation, fiber uniformity, and fiber length. The following statistically significant differences were detected in the combined-site analysis. MON 88701 plants were shorter than the conventional control at the 30 DAP (18.0 vs. 19.2 cm) and at harvest (96.1 vs. 105.0 cm), had increased nodes above white flower at observation 2 (5.5 vs. 5.2), had a decreased seed index (9.4 vs. 10.7 g/100 seed), had increased total seed per boll (29.1 vs. 27.0 seed) and increased mature seed per boll (23.3 vs. 20.1), and had increased fiber strength as compared to the conventional control (30.9 vs. 30.2 g/tex). However, the mean values of MON 88701 were within the reference range for the seven characteristics listed above. Thus, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control. (See Figure VII-1, Step 3, answer "no").

Results of the supplemental evaluation of MON 88701, described above, under the agronomic system in which it is expected to be used (*i.e.*, MON 88701 treated with dicamba and glufosinate herbicides) are provided in Appendix G and further demonstrate that MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See G.12.3).

VII.C.2.1.3. Field Phenotypic and Agronomic Characteristics – Conclusion

The results of the agronomic and phenotypic assessments on MON 88701 from Study 1 and Study 2 demonstrate that the introduction of the dicamba and glufosinate-tolerant traits did not alter MON 88701 compared to the conventional control relating to plant pest/weed potential. Additionally, agronomic and phenotypic assessments of MON 88701 treated with dicamba and glufosinate herbicides were also comparable to the conventional control. Thus, the introduction of the dicamba and glufosinate-tolerant traits into cotton is not likely to result in increased plant pest potential, increased weediness or an altered environmental impact from MON 88701 compared to commercially cultivated cotton.

Table VII-6. Study 2 Combined-Site Comparison of MON 88701 to Conventional Control during 2010 for Phenotypic and Agronomic Characteristics

Phenotypic Characteristic (units)		MON 88701[1] Mean (SE)[3]	Control Mean (SE)	Reference Range[2] Minimum	Reference Range[2] Maximum
Stand Count at 14 DAP[4]	(# in 2 rows per plot)	150.5 (4.2)	155.0 (4.4)	108.4	135.8
Stand Count at 30 DAP	(# in 2 rows per plot)	149.4 (3.9)	152.8 (4.0)	105.8	134.1
Final Stand Count at harvest	(# in 2 rows per plot)	146.3 (4.0)	150.5 (4.3)	110.5	137.7
Plant Height at 30 DAP (cm)		18.0 (1.1)*	19.2 (1.1)	11.4	20.7
Plant Height at harvest (cm)		96.1 (4.2)*	105.0 (4.9)	85.2	121.9
Nodes Above White Flower:	(# of nodes at observation 1)	6.6 (0.2)	6.4 (0.2)	6.0	7.3
	(# of nodes at observation 2)	5.5 (0.3)*	5.2 (0.3)	4.8	5.7
	(# of nodes at observation 3)	4.1 (0.2)	3.8 (0.2)	3.2	4.6
Seedcotton Yield (kg/ha)		3,334.1 (210.2)	3,164.1 (210.8)	2,181.7	3,970.8
Seed Index (g per 100 fuzzy seed)		9.4 (0.2)*	10.7 (0.2)	9.4	12.4
Total Seed per Boll (# per boll)		29.1 (0.4)*	27.0 (0.4)	26.1	30.7
Mature Seed per Boll (# per boll)		23.3 (0.7)*	20.1 (0.8)	14.6	27.0
Immature Seed per Boll (# per boll)		5.8 (0.6)	6.9 (0.6)	2.7	14.4
Weight per Boll (g)		4.9 (0.1)	4.8 (0.1)	4.5	5.9
Fiber Micronaire (mic units)[5]		4.7 (0.1)	4.6 (0.1)	4.2	5.0
Fiber Elongation (%)		6.1 (0.1)	6.2 (0.1)	5.6	8.1
Fiber Strength (g/tex)		30.9 (0.2)*	30.2 (0.2)	30.7	34.0
Fiber Uniformity (%)		83.6 (0.2)	83.4 (0.2)	82.8	84.3
Fiber Length (cm)		2.8 (0.0)	2.8 (0.0)	2.7	3.1

* Indicates a statistically significant difference (α=0.05) between MON 88701 and the conventional control (n = 44).
[1] MON 88701 plots were not treated with dicamba or glufosinate.
[2]Reference range = Minimum and maximum mean values across all 11 sites and eight references from the Study 2 field trial.
[3] SE = standard error.
[4] DAP = days after planting.
[5] Measure of fiber fineness and maturity (expressed in dimensionless micronaire units).

VII.C.2.2. Plant Mapping Characteristics

Plant mapping is a process commonly used by agronomists and breeders to quantify growth and development parameters of a cotton plant, including boll retention (Kerby et al., 2010; Plant and Kerby, 1995). Plant mapping parameters are used to measure crop productivity and are influenced by abiotic and biotic stressors. Plant mapping characteristics (Table VII-7) were evaluated under field conditions to provide USDA-APHIS with a detailed description of MON 88701 boll retention and distribution relative to the conventional control and commercial reference varieties, and to consider differences in context of pest/weed potential.

In addition to the methods discussed in Section VII.C.2.1, 10 plants from each plot in Study 2 were mapped at harvest for the number of mainstem nodes, number of nodes to the first fruiting branch, total number of bolls (sum of first-position, second-position and vegetative bolls), total number of first-position bolls, and total number of vegetative bolls. The percent of first-position bolls relative to total bolls and percent retention of first-position bolls on mainstem fruiting branches were calculated from plant mapping data. The combined-site statistical analysis comparing MON 88701 not treated with either dicamba or glufosinate to the conventional control is summarized below. Results of the individual site data comparisons are presented in Appendix G.13.1. Also the experimental methods and detailed results from the supplemental analyses comparing MON 88701 treated with dicamba and glufosinate herbicides to the conventional control are presented and discussed in Appendix G.13.2.

In the combined-site analysis of plant mapping parameters (Table VII-7), no statistically significant differences were detected between MON 88701 and the conventional control for number of mainstem nodes per plant, number of nodes to first fruiting branch, total number of bolls per plant, vegetative bolls per plant, percent retention of first-position bolls and percent first-position bolls (relative to total bolls). The mean value for first-position bolls per plant was higher in MON 88701 than the conventional control (5.2 vs. 4.6) (Table VII-7). However, the mean value for first-position bolls per plant was within the reference range. Furthermore, similar results of the plant mapping evaluation of dicamba and glufosinate-treated MON 88701, the agronomic system in which MON 88701 is expected to be used, were observed (See Appendix G.13.2; Table G-18). Thus, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control. (See Figure VII-1, Step 3, answer "no").

Table VII-7. Study 2 Combined-Site Comparison of MON 88701 to Conventional Control during 2010 for Plant Mapping Characteristics

Phenotypic Characteristic (units)	MON 88701[1] Mean (SE)[3]	Control Mean (SE)	Reference Range[2] Minimum	Reference Range[2] Maximum
Mainstem Nodes (# per plant)	18.1 (0.4)	18.2 (0.4)	16.0	21.6
Nodes to First Fruiting Branch (# per plant)	5.2 (0.2)	5.5 (0.2)	4.2	7.6
Total Bolls[4] (# per plant)	9.8 (0.6)	9.0 (0.7)	8.6	13.4
Total First-Position Bolls (# per plant)	5.2 (0.3)*	4.6 (0.3)	2.9	6.3
Total Vegetative Bolls (# per plant)	2.0 (0.6)	1.9 (0.6)	0.7	5.0
% Retention of First-Position Bolls (per plant)	42.1 (2.5)	38.6 (2.6)	21.2	53.5
% First-Position Bolls relative to total bolls (per plant)	57.5 (2.2)	56.5 (2.1)	36.0	59.6

* Indicates a statistically significant difference (α=0.05) between MON 88701 and the conventional control (n = 44).
[1] MON 88701 plots were not treated with dicamba or glufosinate.
[2] Reference range = Minimum and maximum mean values among eight conventional commercial reference varieties.
[3] SE = standard error.
[4] Total Bolls = number of first-position bolls + number of second-position bolls + number of vegetative bolls.

VII.C.2.3. Environmental Interaction Characteristics

USDA-APHIS considers the environmental interactions of the biotechnology-derived crop compared to its conventional counterpart to determine the potential for increased plant pest characteristics. Evaluations of environmental interactions were conducted as part of the plant characterization for MON 88701. In the 2010, US field trials conducted for evaluation of phenotypic and agronomic characteristics of MON 88701, data were also collected on plant response to abiotic stress (*i.e.*, drought, wind, nutrient deficiency, etc.), disease damage, arthropod-related damage, and arthropod abundance (Appendix G; Tables G-20 through G-29). These data were used as part of the environmental analysis (Section IX) to assess plant pest potential and provide an indication of potential effects of MON 88701 on non-target organisms (NTOs) compared to the conventional control. The results of the field evaluations showed that the dicamba and glufosinate-tolerance traits did not unexpectedly alter the assessed environmental interactions of MON 88701 compared to the conventional control. The lack of significant biologically meaningful differences in plant response to abiotic stress, disease damage, arthropod-related damage, and pest- and beneficial-arthropod abundance supports the conclusion that the introduction of the dicamba and glufosinate-tolerance traits are unlikely to result in increased plant pest potential or an altered environmental impact from MON 88701 compared to commercial cotton.

VII.C.2.3.1. Study 1 Environmental Interactions of MON 88701

MON 88701 was compared to the conventional control for qualitative and quantitative environmental interactions in Study 1 (See Section VII.C.2.1.). Qualitative assessments were conducted at 15 sites and included plant response to abiotic stressors, disease damage, and arthropod damage. The assessments were conducted four times during the growing season on all plots (4 time points x 4 plot replications = 16 data points per assessment). The first assessment was made at approximately 30 days after planting and the three subsequent assessments at approximately 30 day intervals thereafter.

Plant response to abiotic stressors, disease damage, and arthropod damage were assessed at natural levels (no artificial infestation or imposed abiotic stress); therefore these levels typically varied between observations at a site and among sites. Plant response to abiotic stress, and disease damage and arthropod damage data were collected from each plot using a 0 – 9 scale of increasing severity of observed damage for each stressor. This scale was utilized to allow for the evaluation of the wide variety of potential abiotic stressor, disease damage, and arthropod damage symptoms potentially occurring across the season and across sites. Due to the non-specific nature of the scale used, the data were not statistically analyzed but rather were placed into one of the following categories: none (0), slight (1-3), moderate (4-6), or severe (7-9). MON 88701 and conventional control cotton were considered different in plant response to stressors if the range of injury symptoms across all four replications did not overlap between MON 88701 and the conventional control. Any observed differences between the MON 88701 and conventional control were assessed for biological significance in the context of the range of the commercial reference varieties, and for consistency in other observations and sites.

In addition to the qualitative assessment, quantitative arthropod assessments were conducted at five sites and included thrips damage, heliothine damage, and pest- and beneficial-arthropod abundance. Thrips damage was assessed three times (approximately 14, 21, and 28 DAP) during the growing season, heliothine damage was assessed four times (approximately 45, 60, 75, and 90 DAP) during the growing season, and arthropod abundance was assessed from collections performed four times (approximately 30, 60, 90, and 120 DAP) during the growing season.

Thrips damage was quantitatively assessed in each plot from 10 randomly selected plants using the arthropod-specific 0–5 rating scale of increasing severity. Heliothine damage was assessed quantitatively by recording the total number of fruiting bodies (flower buds, flowers, and bolls), number of damaged fruiting bodies and number of live larvae on the top 7 nodes from 10 randomly selected plants of each plot. These numerical data along with the quantitative arthropod abundance data were subjected to statistical analysis.

VII.C.2.3.1.1. Qualitative Assessment Results - Study 1

In an individual site assessment of qualitative data (Tables VII-8, G-20 through G-22), no differences were observed between MON 88701 and the conventional control for any of the 169 comparisons for plant response to abiotic stressors, including compaction, drought/dry, flood, hail, heat, nutrient deficiency, wet soil/ excess precipitation, and wind damage. Also, no differences were observed between MON 88701 and the conventional control for any of the 170 comparisons for the assessed diseases, including anthracnose, *Ascochyta* leafblight, bacterial blight, boll rot, cotton leaf rust, damping off, *Fusarium* wilt, leaf spots, *Pythium*, reniform nematode, *Rhizoctonia*, root-knot nematode, *Thielaviopsis*, and *Verticillium* wilt. Finally, no differences were observed between MON 88701 and the conventional control for any of the 159 comparisons for the assessed arthropods, including aphids, beet armyworms, cut worms, fall armyworms, fleahoppers, grasshoppers, heliothines, southern corn rootworm beetles, soybean loopers, spider mites, stink bugs, tarnished plant bugs, thrips, and whiteflies. Since no differences were observed between MON 88701 and the conventional control for plant response to abiotic stressors, disease damage, and arthropod-related damage in multiple environments, the assessed results support the conclusion that MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Section VII.B.1.1.).

Table VII-8. Study 1 Summary of Qualitative Environmental Interactions Assessments Including MON 88701 Response to Abiotic Stress, Disease, and Arthropod Damage during 2010

Stressor	Number of Observations Across All Sites	Number of Observations with No Observed Differences Between MON 88701 and the Conventional Control
Abiotic stressors	169	169
Disease damage	170	170
Arthropod-related damage	159	159
Total	498	498

Note: The experimental design was a randomized complete block with four replications (n = 60).

VII.C.2.3.1.2. Quantitative Assessment Results - Study 1

In the combined-site analysis of thrips damage data (Table VII-9), no statistically significant differences were detected between MON 88701 and the conventional control. There were no biological differences in thrips damage that would contribute to increased pest potential of MON 88701 compared to the conventional control (See Section VII.B.1.1.).

Table VII-9. Study 1 Combined-Site Comparison of MON 88701 to Conventional Control during 2010 for Assessment of Thrips Damage

Observation	MON 88701 (SE)[1]	Control (SE)	Reference range
1	0.5 (0.1)	0.4 (0.1)	0.0 – 1.2
2	0.1 (0.0)	0.1 (0.0)	0.0 – 0.2
3	0.0 (0.0)	0.1 (0.0)	0.0 – 0.5

Note: The experimental design was a randomized complete block design with four replications (n = 20). No statistically significant differences (α=0.05) were detected between MON 88701 and the conventional control.
[1]SE= standard error.

In the combined-site analysis of heliothine damage data (Table VII-10), no statistically significant differences were detected between MON 88701 and the conventional control for percent damaged fruiting bodies and the number of live larvae. Thus, there is no biological difference in heliothine damage that would contribute to increased pest potential of MON 88701 compared to the conventional control (See Section VII.B.1.1.).

Table VII-10. Study 1 Combined-Site Comparison of MON 88701 to Conventional Control during 2010 for Quantitative Assessment of Heliothine Damage

Observation	Percent Damaged Fruiting Bodies			# of Live Larvae[1]		
	MON 88701 (SE)[2]	Control (SE)	Reference Range	MON 88701 (SE)	Control (SE)	Reference Range
1	2.8 (0.9)	1.8 (0.8)	0.0 – 8.7	0.1 (0.1)	0.0 (0.0)	0.0 – 0.2
2	5.3 (3.0)	6.3 (2.8)	1.2 – 28.1	0.2 (0.1)	0.1 (0.0)	0.0 – 0.5
3	3.7 (0.7)	2.6 (0.5)	1.2 – 5.2	0.1 (0.0)	0.1 (0.0)	0.0 – 0.1
4	6.3 (1.9)	6.9 (1.8)	2.6 – 12.1	0.1 (0.0)	0.1 (0.0)	0.0 – 0.3

Note: No statistically significant differences (α=0.05) were detected between MON 88701 and conventional control (n = 20).
[1] Number of immature heliothines.
[2] SE = standard error.

For arthropod abundance, a total of 178 comparisons were made between MON 88701 and the conventional control for the following pest- and beneficial-arthropods: aphids, cabbage loopers, fall armyworms, fleahoppers, heliothines, southern armyworms, stink bugs, tarnished plant bugs, thrips, white flies, big eyed bugs, braconids, lacewings, ladybird beetles, Damsel bugs, *Orius* spp., and spiders (Araneae) (Tables G-25 and G-26). No statistically significant differences were detected between MON 88701 and the conventional control for 173 out of 178 comparisons, including 89 pest arthropod comparisons and 89 beneficial arthropod comparisons.

The five differences detected between MON 88701 and the conventional control included two differences for pest arthropods and three differences for beneficial arthropods (Tables G-25 and G-26). In the pest arthropod assessment, MON 88701 had lower abundance than the conventional control for stink bugs (0.3 vs. 1.8 per plot) and for tarnished plant bugs (0.5 vs. 2.0 per plot) in Collection 4 at the LABU site. For tarnished plant bugs, the mean abundance value for MON 88701 was within the reference range. For stink bugs, the mean abundance value for MON 88701 was outside the reference range. However, the statistical differences detected in stink bugs abundance were not consistent across collections or sites (Table G-25).

In the beneficial arthropod assessment, MON 88701 had increased abundance compared to the conventional control (Table G-26) for Damsel bugs in Collection 2 at the GACH site (6.0 vs. 2.3). MON 88701 had lower abundance than the conventional control for *Orius* spp. in Collection 2 (0.0 vs. 1.5 per plot) and collection 3 (0.5 vs. 2.8 per plot) at the ARAU site. The mean abundance value for MON 88701 was within the reference ranges for the differences detected for Damsel bugs The mean abundance values for *Orius* spp. in Collection 2 and collection 3 at the ARAU site were outside their respective reference range. However, the differences detected for *Orius* spp. were not consistently detected across collections or sites (Table G-26).

Since the arthropod differences detected were not consistently observed at other collections and sites, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Section VII.B.1.1.).

VII.C.2.3.2. Study 2 Environmental Interactions of MON 88701

MON 88701 was compared to the conventional control for qualitative environmental interactions in Study 2 (See section VII.C.2.1.). Only qualitative assessments were conducted at all 11 sites and included plant response to abiotic stressors, arthropod damage, and disease damage. The observations of plant response to abiotic stressors, disease damage, and arthropod damage were performed four times during the growing season at each site on all plots (4 replications). The first observation was made at approximately 30 days after planting and the three subsequent observations at approximately 30 day intervals thereafter. (Section VII.C.2.3.1).

VII.C.2.3.2.1 Qualitative Assessment Results - Study 2

In an individual site assessment for Study 2 qualitative data (Table VII-11, G-27, G-28 and G-29), no differences were observed between MON 88701 and the conventional control for any of the 127 comparisons for plant response to abiotic stressors, including compaction, drought (dry), flood, hail damage, heat, nutrient deficiency, wet soil (excess precipitation), and wind damage. Also, no differences were observed between MON 88701 and the conventional control for any of the 129 comparisons for the assessed diseases, including anthracnose, ascochyta leaf blight, bacterial blight, boll rot, cotton leaf rust, damping off, *Fusarium* wilt, leaf spots, *Pythium*, reniform nematode, *Rhizoctonia*, root-knot nematode, thielaviopsis, and *Verticillium* wilt. Finally, no differences were observed between MON 88701 and the conventional control for any of the 129 comparisons for the assessed arthropods, including aphids, beet armyworms, cabbage loopers, cut worms, fall armyworms, fleahoppers, grasshoppers, heliothines, southern corn rootworm beetle, soybean loopers, spider mites, stink bugs, tarnished plant bugs, thrips and white flies. Since no differences were observed between MON 88701 and the conventional control for plant response to abiotic stressors, disease damage, and arthropod-related damage in multiple environments, the assessed results are similar to those in Study 1 and support the conclusion that the biotechnology-derived traits in MON 88701 are unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Section VII.B.1.1.).

Table VII-11. Study 2 Summary of Qualitative Environmental Interactions Assessments Including MON 88701 Response to Abiotic Stress and Disease and Arthropod-related Damage during 2010

Stressor	Number of Observations Across All Sites	Number of Observations with No Observed Differences Between MON 88701 and the Conventional Control
Abiotic stressors	127	127
Disease damage	129	129
Arthropod-related damage	129	129
Total	385	385

Note: The experimental design was a randomized complete block with four replications (n = 60

VII.C.2.3.2.2. Plant Mapping as an Indicator of Plant Response to Environmental Stress

Final boll retention and distribution, as reflected in the plant mapping data, can provide an indication of the effect that abiotic and biotic stressors had on a cotton plant because squares and early bolls tend to abort if the plant experiences stress ([redacted], 1982; Kerby et al., 2010; University of California, 1996). For example, if plants experienced severe stress during early flowering, this could result in fewer bolls on the lowermost fruiting branches compared to unstressed plants. If plant map results are similar between two cotton lines this usually indicates that plants responded to stress in a similar manner. Within a study location and based on the proximity of plots within a location, it can be concluded that all plots would be subjected to similar stressors.

As previously indicated, there were no differences in plant mapping parameters between MON 88701 not treated with dicamba or glufosinate herbicides and the conventional control that would be indicative of a differential plant response to abiotic or biotic stressors (Study 2, Section VII.C.2.2, Table VII-7). Similar results were observed for MON 87701 plots treated with dicamba and glufosinate (Table G-18). Thus, since all Study 2 plots would be subjected to similar stressors and since MON 88701 treated and not treated with dicamba or glufosinate herbicides had similar plant map results, each compared to the conventional control (See Tables VII-7 and G-18); it can be concluded that both responded to stressors in a similar manner. Results showed that only the mean number of first-position bolls was significantly different in both comparisons of MON 88701 not treated compared to the conventional control (5.2 vs. 4.6, respectively) and MON 88701 treated compared to the conventional control (5.2 vs. 4.6, respectively). Both of the mean values of the number of first-position bolls in MON 88701 were within the reference range. Therefore, these data support the conclusion that the biotechnology-derived traits in MON 88701 are unlikely to have increased plant pest potential, increased

weediness, or an adverse environmental impact compared to commercially cultivated cotton.

VII.C.2.3.3. Conclusions - Qualitative and Quantitative Environmental Interactions

The results of the qualitative and quantitative data of MON 88701 from Study 1 and qualitative data from Study 2 showed that the dicamba and glufosinate-tolerance traits did not unexpectedly alter the assessed environmental interactions of MON 88701 compared to the conventional control. The lack of significant biological differences in plant responses to abiotic stress, disease damage, arthropod-related damage, thrips damage, heliothine damage, and pest- and beneficial-arthropod abundance for MON 88701 supports the conclusion that the introduction of the dicamba and glufosinate-tolerance traits are unlikely to result in increased plant pest potential, increased weediness or an altered environmental impact from MON 88701 compared to commercially cultivated cotton, irrespective of whether or not dicamba and glufosinate herbicide treatments were applied.

VII.C.3. Pollen Characteristics

USDA-APHIS considers the potential for gene flow and introgression of the biotechnology-derived trait(s) into other cotton varieties and wild relatives to assess the potential for increased weedy or invasive characteristics of the receiving species. Pollen morphology and viability information are pertinent to this assessment and, therefore, were assessed for MON 88701. In addition, characterization of pollen produced by MON 88701 and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 88701 compared to the conventional control.

The purpose of this evaluation was to assess the morphology and viability of pollen collected from MON 88701 compared to that of the conventional control. Pollen was collected from MON 88701, the conventional control, and four commercial reference varieties grown under similar agronomic conditions in Crittenden County, Arkansas (ARPR). The field trial was arranged in a randomized complete block design with four replications. Five flowers (subsamples) were collected from each plot; pollen was extracted from each flower and stained with Alexander's stain (Alexander, 1980). Pollen viability was evaluated for each subsample and pollen grain diameter was evaluated for ten representative viable pollen grains per subsample. General morphology of the pollen was observed for each subsample. MON 88701 was compared to the conventional control for percentage viable pollen and pollen diameter. A reference range was calculated from the minimum and maximum mean values of the commercial reference varieties to provide pollen viability and pollen diameter values representative of commercial cotton (See Appendix H).

No statistically significant differences (α=0.05) were detected between MON 88701 and the conventional control for percentage viable pollen or pollen grain diameter (Table VII-12). Furthermore, no visual differences in general pollen morphology were observed between MON 88701 and the conventional control. These results demonstrate that the

introduction of the dicamba and glufosinate-tolerance traits did not alter the overall morphology or pollen viability of MON 88701 compared to the conventional control (See Figure VII-1, Step 2, answer "no"). The pollen characterization data contribute to the detailed phenotypic description of MON 88701 compared to the conventional control. The results support an overall conclusion that MON 88701 is not different than the conventional control in terms of plant pest or weed characteristics and is no more likely to pose a plant pest risk than commercially cultivated cotton.

Table VII-12. MON 88701 Compared to the Conventional Control during 2010 for Pollen Characteristics

	MON 88701	Conventional Control	Reference Range[1]	
Pollen Characteristic	Mean (S.E.)[2]	Mean (S.E.)	Minimum	Maximum
Viability (%)	97.8 (0.46)	97.9 (0.40)	96.0	98.2
Diameter (µm)	95.2 (1.49)	92.2 (1.47)	94.0	95.2

The experimental design was a randomized complete block design with four replications (n = 4). No significant differences were detected between MON 88701 and the conventional control (α=0.05) using analysis of variance (ANOVA).

[1] Reference ranges = Minimum and maximum values of four commercial reference varieties.

[2] S.E. = Standard Error.

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

Domesticated cotton lacks characteristics that are commonly associated with plants that are considered weeds (e.g. seed dormancy, seed dispersal mechanisms, ability to compete with and displace native vegetation). An extensive and robust set of information and data were used to assess whether the introduction of the dicamba and glufosinate-tolerant traits altered the plant pest potential of MON 88701 compared to the conventional control. These assessments included five general data categories: 1) seed dormancy and germination characteristics; 2) agronomic and phenotypic characteristics; 3) plant mapping characteristics; 4) observations of abiotic stress response, disease damage, arthropod related damage, and pest- and beneficial-arthropod abundance; and 5) pollen characteristics. Results from these assessments comparing MON 88701 and the conventional control demonstrate that MON 88701 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stressors, diseases, or arthropods, or characteristics that would confer a plant pest risk or significant environmental impact compared to conventional cotton. Therefore, based on the results of multiple assessments discussed above, MON 88701 is comparable to commercially cultivated cotton, and is no more likely to pose a plant pest/weediness risk or have a significant environmental impact.

VIII. U.S. AGRONOMIC PRACTICES

VIII.A. Introduction

As part of the plant pest assessment required by 7 CFR § 340.6(c)(4), impacts to agricultural and cultivation practices must be considered. This section provides a summary of current agronomic practices in the U.S. for producing cotton and is included in this petition as a baseline to assess possible impacts to agricultural practices due to the cultivation of MON 88701. Discussions include cotton production, seed production, plant growth and development, general management practices during the season, management of insects, diseases and weeds, cotton rotational crops, and volunteer cotton management. Information presented in the previous section demonstrated that MON 88701 is no more susceptible to diseases or pests than commercially cultivated cotton. Additionally, data presented in Section VII show that, with the exception of tolerances to both dicamba and glufosinate herbicides, MON 88701 is phenotypically equivalent to commercially cultivated cotton. Thus, there are no changes to the inputs needed for MON 88701, and no likely impacts to the majority of the agronomic practices employed for the production of cotton. Agronomic practices that maybe influenced from the deregulation of MON 88701 are discussed.

Cotton production in the U.S. is limited primarily by climate. Cotton is a woody, warm-season perennial plant that is planted in 17 states across the southern U.S. Aside from temperature, the most influential climatic factor impacting cotton agronomic practices is moisture. Rainfall requirements and patterns are a major determinant of the cotton production practices adopted in both dryland and irrigated cotton. The length of the season may vary between cotton production regions, but the production cycle and production practices used are fairly consistent among geographic regions and between the upland and Pima cotton types. 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 cotton.

Annual and perennial weeds are a serious problem and must be managed in order to maximize cotton yield and quality. Weeds compete with cotton for water, nutrients, and light, resulting in reduced cotton lint yields and lint quality when left uncontrolled. Weed species in cotton vary from region to region and from state to state, but the economic thresholds of cotton require some form of weed management practice on all cotton acreage. Weed management practices include mechanical tillage, crop rotations, cultural practices (*e.g.*, planting clean seed, cleaning tillage and harvesting equipment), and herbicide application. Numerous selective herbicides are utilized for preplant, preemergence, and postemergence control of annual and perennial weeds in cotton. Approximately 97% of the cotton acreage in the U.S. receives a herbicide application. Herbicide-tolerant cotton is currently grown on 78% of U.S. cotton acres (Brookes and Barfoot, 2012) and glyphosate-tolerant cotton weed control systems have become the standard program for weed management in cotton since commercial introduction of glyphosate-tolerant cotton in 1997 (Brookes and Barfoot, 2012). Glyphosate-tolerant cotton has facilitated the growth of conservation tillage systems in cotton production which has resulted in reduced soil erosion, reduced fuel and labor costs, improved water

quality, and conserved soil moisture. Herbicides can replace the need for preplant tillage for weed control in no-tillage production systems. Insect pests, diseases, and nematodes are also common and continuous threats to cotton production and integrated pest management programs must be implemented to prevent yield losses due to these pests.

Volunteer cotton (*i.e.*, cotton plants that have germinated and emerged unintentionally in a subsequent crop) is not considered a significant problem in rotational crops primarily because mechanical and chemical control methods are available to manage the occasional volunteer cotton plant. Preplant tillage generally destroys volunteer cotton plants prior to planting rotational crops. Volunteer cotton is generally more of a problem in no-till cotton because of the lack of preplant tillage, but herbicides are available for control of volunteer cotton in rotational crops. Given that MON 88701 is agronomically, phenotypically, and ecologically comparable to commercially cultivated cotton, the introduction of MON 88701 in the cotton production system is expect to have no impact on the management of cotton volunteer plants in rotational crops such as corn, soybean, sorghum, and wheat. The numerous control measures that are effective on conventional and glyphosate-tolerant volunteer plants will continue to be effective on volunteer MON 88701 plants if they arise. See Section VIII.H.1 for additional information on control of MON 88701 volunteers.

As shown in Sections VI and VII, with the exception of the tolerances to both dicamba and glufosinate herbicides, no biologically meaningful differences were observed in composition, phenotype, or environmental interactions between MON 88701 and commercially cultivated cotton. Moreover, herbicide-tolerant cotton is currently grown on 78% of U.S. cotton acres (Brookes and Barfoot, 2012). Therefore, it is anticipated that commercialization of MON 88701 in the U.S. is not likely to impact current cotton cultivation and/or agronomic practices, beyond the intended benefits of effective management of common and troublesome weeds, including herbicide-resistant weeds.

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

VIII.B.1. Cotton Production

The majority of the value of the producer's cotton crop is based on the quality and quantity of the lint produced, and with the exception of contracted acres for planting seed production. Little consideration is given by growers to the disposition of the cottonseed and its by-products. Most of the world's cotton production (116.40 million bales annually) is grown in China (30.5 million bales), India (26.4 million bales), United States (18.1 million bales), Pakistan (8.6 million bales) and Brazil (9.0 million). Figures are from the 2010/2011 cotton season (USDA-FAS, 2012). In 2010/2011, the U.S. supplied over 14 million bales of the world's cotton exports, accounting for approximately 40% of the total world export market for cotton (USDA-FAS, 2011). China, Bangladesh, Indonesia, and Turkey are major importers of cotton. The largest customers for U.S. cotton are Asian countries and Mexico, due to the prevalence of textile manufacturing (NCCA, 2010). Cottonseed production currently results in approximately 10% of the world's oilseed production (USDA-FAS, 2010), and is exceeded by soybean (58%) and rapeseed (13%).

Gossypium hirsutum (upland cotton) cultivars account for more than 90% of the world's annual cotton crop and 97% of the U.S. cotton production (Smith and Cothren, 1999; USDA-NASS, 2011e). *G. barbadense*, known as extra-long staple, Pima, or Egyptian cotton, is also grown in the U.S, which accounts for approximately 3% of the acreage in the U.S. (USDA-NASS, 2012c). The long, strong, fine fibers produced by Pima are ideal for specialized uses, but due to the geographic limitation for optimum production it is economically less viable than the *G. hirsutum* cultivars in the U.S. Pima cotton requires a longer growing season than upland cotton, and production is limited to the Southwestern states.

Cotton is a crop that produces two commodities: fiber and seed. The modern cotton gin has enhanced the value of cotton commodities by separating the fiber from the seed and by removing foreign matter, while preserving the inherent qualities of the fiber and seed (Smith and Cothren, 1999). The fiber is the more valuable product of the crop, normally accounting for approximately 85% of the value. For every 100 pounds of fiber produced by the cotton plant, it also produces about 162 pounds of cottonseed (NCCA, 2010). Cottonseed is crushed for oil and meal used in both food products and in livestock feed.

Cotton (*Gossypium* spp.) is grown in the U.S. across southern states where the climate is warmer and the season is longer (Figures VIII-1 and VIII-2). The total U.S. cotton acreage in the past 10 years has varied from approximately 9.15 to 15.77 million planted acres, with the lowest acreage recorded in 2009 and the highest in 2001 (Table VIII-1). Average cotton yields have varied from 632 to 879 pounds per acre over this same time period. Total annual cotton production ranged from 12.19 to 23.89 million bales (480 pounds/bale) over the past ten years. The variations observed in cotton acreage and production is driven by current market conditions, rather than agronomic considerations. According to data from USDA-NASS (USDA-NASS, 2011b), cotton was planted on approximately 11 million acres in the U.S. in 2010, producing approximately 18 million bales of cotton (Table VIII-1). The value of cotton production reached $7.32 billion in the U.S. in 2010 (USDA-NASS, 2011b).

U.S. cotton production is divided into the following four major cotton growing regions, which span the southern and southwestern states: Southeast region (AL, FL, GA, NC, SC, and VA), Midsouth region (AR, LA, MS, MO, and TN), Southwest region (KS, NM, OK, and TX), and West region (AZ and CA) (Table VIII-2). Cotton planting and production figures for these regions in 2010 are shown in Table VIII-2 and discussed below (USDA-NASS, 2011e). Approximately 5.6 million acres of cotton were planted in Texas, representing about 51% of the total U.S. cotton acres. Texas produced 8.1 million bales (480 pounds/bale) of cotton, which represents approximately 44% of the U.S. cotton production. The second largest production state for cotton was Georgia with approximately 12% of U.S. cotton production. Average cotton yields across the four cotton growing regions ranged from 727 to 1416 pounds cotton lint per acre, with the highest yields in the West with full irrigation, and the lowest yields in areas such as Alabama, Oklahoma, and Texas, where little to no irrigation is employed (Table VIII-2). The average cotton yield across all regions is 821 pounds cotton lint per acre. The value of the cotton lint production among the four regions ranged from $0.86 billion in the West region to $3.35 billion in the Southwest region. The total value of the cottonseed

production in the U.S. in 2010 was $1 billion with the value among the regions ranging from $134 million in the West region to $461 million in the Southwest region.

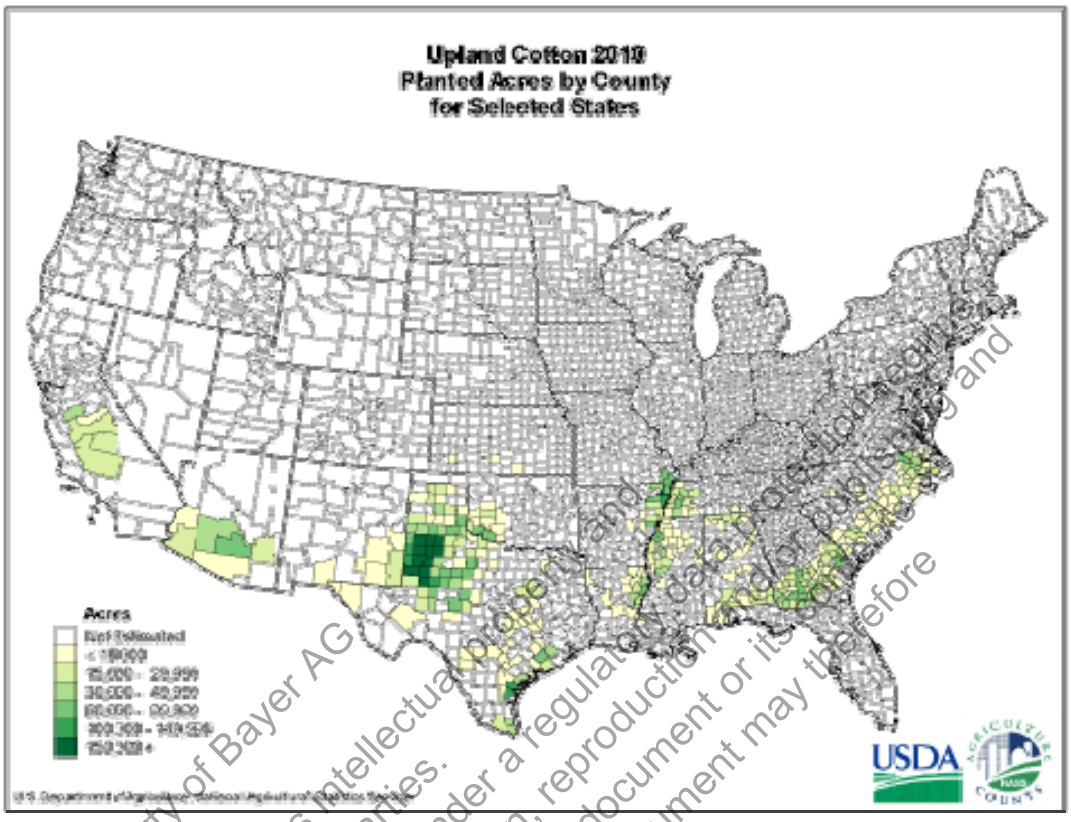


Figure VIII-1. Planted Upland Cotton Acres by County in the U.S. in 2010
(USDA-NASS, 2012a)

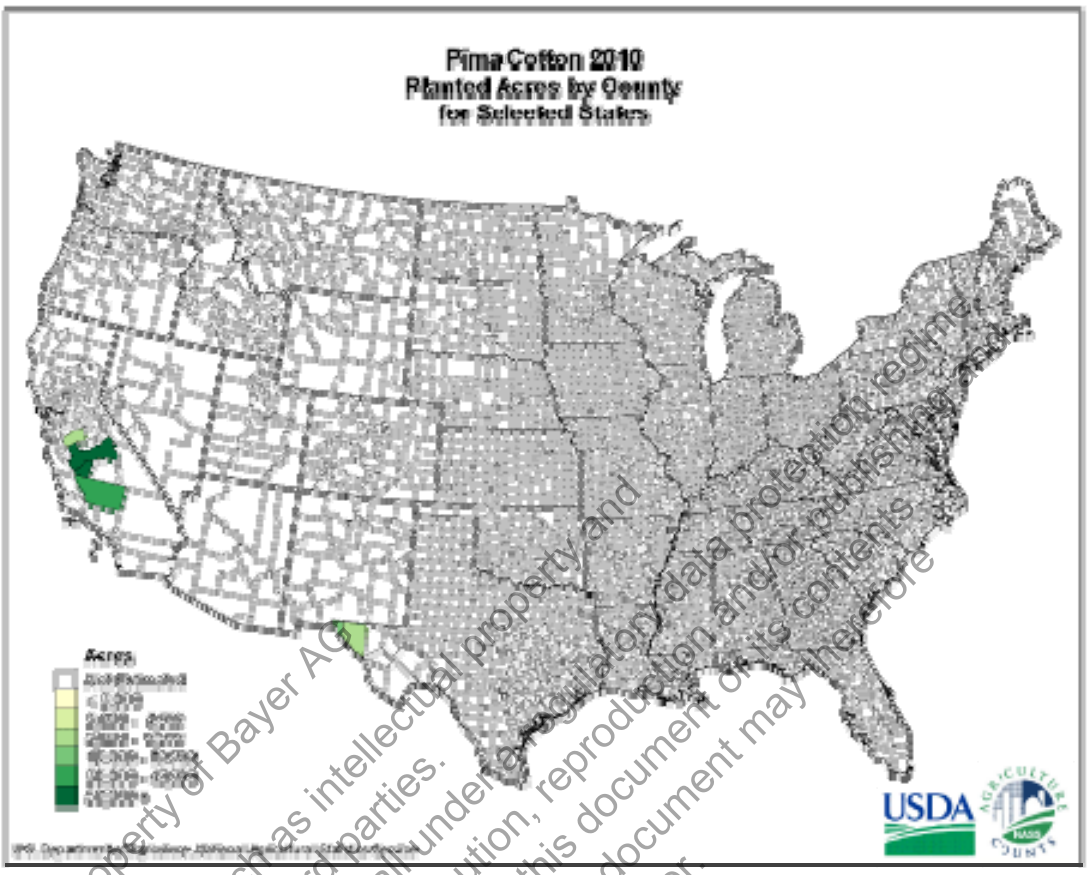


Figure VIII-2. Planted Pima Cotton Acres by County in the U.S. in 2010
(USDA-NASS, 2012b)

Table VIII-1. Cotton Production in the U.S., 2000-2010[1]

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (lbs/acre)	Total Production (480 lb bales)	Value (billions $)
2010	10,973	10,707	821	18,314,500	7.318
2009	9,150	7,691	777	12,187,500	3.788
2008	9,471	7,569	813	12,815,300	3.021
2007	10,872	10,489	879	19,206,900	5.653
2006	15,274	12,732	814	21,587,800	5.013
2005	14,245	13,803	831	23,890,200	5.695
2004	13,659	13,057	855	23,250,700	4.853
2003	13,480	12,003	730	18,255,200	5.517
2002	13,958	12,417	665	17,208,600	3.777
2001	15,769	13,828	705	20,302,800	3.122
2000	15,517	13,053	632	17,188,300	4.260

[1] (USDA-NASS, 2011b)

Table VIII-2. U.S. Cotton Production by Region and State in 2010[1]

Region/State	Acres Planted (thousands)	Acres Harvested (thousands)	Average Yield (pounds/acre)	Total Production (thousand bales)	Cotton Lint $ Value (thousands)	Cottonseed $ Value (thousands)
Southeast Region						
Alabama	340	337	684	480	199,066	20,856
Florida	92	89	809	150	54,792	5,720
Georgia	1,330	1,320	811	2,230	926,966	91,120
North Carolina	550	545	854	970	338,957	44,992
South Carolina	202	201	872	365	136,656	16,756
Virginia	83	82	685	117	46,051	6,300
Region Totals	**2,597**	**2,574**	**804**	**4312**	**1,702,488**	**185,744**
Midsouth Region						
Arkansas	545	540	1,049	1,180	395,914	71,400
Louisiana	255	250	864	450	174,960	24,024
Mississippi	420	415	983	850	308,856	44,616
Missouri	310	308	1,068	685	226,214	40,630
Tennessee	390	387	843	680	275,482	42,180
Region Totals	**1,920**	**1,900**	**971**	**3,845**	**1,381,426**	**222,850**
Southwest Region						
Kansas	51	49	784	80	34,675	3,712
New Mexico	50	49	1,084	110	46,721	7,215
Oklahoma	285	270	738	415	180,276	20,727
Texas	5,567	5,367	723	8,082	3,083,472	429,814
Region Totals	**5,953**	**5,734**	**727**	**8,687**	**3,345,144**	**461,468**
West						
Arizona	198	196	1,460	595	246384	46,200
California	306	303	1,388	876	610042	87,599
Region Totals	**504**	**499**	**1,416**	**1,471**	**856,426**	**133,799**
U.S. Total	**10,973**	**10,707**	**821**	**18,315**	**7,317,704**	**1,003,861**

[1] (USDA-NASS, 2011e; 2011c)

VIII.B.2. Cotton Seed Production

Standardized seed production practices are responsible for maintaining high-quality seed stocks, which is essential for U.S. agriculture. The value of seed quality (including genetic purity, vigor, and absence of weed seed, seed-borne diseases, and inert materials, such as dirt) are a major factor impacting crop yield potential. States developed seed laws and certification agencies to ensure that purchasers who received certified seed could be assured that the seed met established seed quality standards (Bradford, 2006). The federal government passed the U.S. Federal Seed Act of 1939 to recognize seed certification and the establishment of official certifying agencies. Regulations first adopted in 1969 under the Federal Seed Act recognize land history, field isolation, and varietal purity standards for foundation, registered, and certified seed. Under international agreements such as the Organisation for Economic Co-operation and Development (OECD) system, the U.S. and other countries mutually recognize minimum seed quality standards (Bradford, 2006). The Association of Official Seed Certifying Agencies (AOSCA) represents state and private seed certification organizations in the U.S., and includes international member countries in North and South America, Australia, and New Zealand.

Cotton seed is separated into four seed classes: 1) breeder; 2) foundation; 3) registered; and 4) certified (AOSCA, 2012). Breeder seed is seed directly controlled by the originating or sponsoring plant breeding organization or firm. Foundation seed is first-generation seed increased from breeder seed and is handled in a manner to maintain specific levels of varietal purity and identity. Registered seed is the progeny of foundation seed that is handled to maintain satisfactory varietal purity and identity. Certified seed is the progeny of breeder, foundation or registered seed, and is typically two generations removed from foundation seed. While not all cotton seed sold to growers is officially certified, commercial cotton seed sold and planted for typical cotton 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.

The majority of the cotton seed is produced in Texas with significant quantities produced in Arizona, Arkansas, California, and Mississippi (McDonald and Copeland, 1997). The entire seed production process at the majority of the seed companies uses International Organization for Standardization (ISO) certification standards and therefore include internal and external audits (ISO, 2009). ISO standards ensure desirable characteristics of seeds and services, such as quality, safety, reliability, and efficiency. The ISO standards represent an international consensus on good management practices with the aim of ensuring that the organization can consistently deliver excellent products or services. The standards must meet the customer's requirements, applicable seed regulatory requirements, and continually improve the process and process control systems (ISO, 2009). Agronomic practices for producing cotton seed are similar to commercial cotton production. However, increased management is needed in certain agronomic practices (*e.g.*, fertility, water management, cultivation, use of plant growth

regulators, etc.) to produce seed with high quality, high germination rates, and high genetic purity.

After harvest and the ginning and delinting processes, commercially certified cotton seed must meet state and federal seed standards and labeling requirements. AOSCA standards for certified cotton seed are as follows: 98% pure seed (minimum), 2% inert matter (maximum), 0.02% weed seed (maximum), 0.3% other crop seeds (maximum), and 70% germination (minimum) (AOSCA, 2009). The cotton seed industry historically sets a minimum of 80% germination for labeling purposes. State seed certification standards vary slightly from state to state and can be more restrictive than the seed standards of AOSCA.

When deregulated, MON 88701 seed will be produced in the same manner as commercially certified cotton seed, such that it will meet all state and federal seed standards and labeling requirements.

VIII.C. Production Management Considerations

VIII.C.1. Pre-Season

Production decisions regarding crop rotation, tillage system, soil fertility, variety selection, and row spacing need to be made well in advance of planting the cotton crop. Many of the decisions in this area are made prior to or immediately after harvest of the previous crop. The rotation of cotton with other crops should be an integral part of a farm management program. Ideally, cotton should be rotated with other crops on a regular basis to maintain soil productivity and reduce the incidence of various weeds, insect pests or diseases (Hake et al., 1996d). However, production costs, relative rate of return, and the current market conditions will dictate which crops to rotate with cotton or whether to grow continuous cotton. See Section VIII.H for additional details on crop rotation practices in cotton.

Tillage has been an integral part of production agriculture and is synonymous with seedbed preparation. The primary purposes of preplant tillage are to incorporate residue from the previous crop, reduce wheel traffic compaction from the previous season, improve water filtration and soil aeration, control weeds, loosen the soil for root penetration, and provide a suitable environment for the planting and germination of cottonseed (Hake et al., 1996d). Decreased profitability in cotton production, as well as soil erosion concerns, have increased interest in conservation tillage systems. The benefits of conservation tillage or no-till systems are well documented and include reduced soil erosion, reduced fuel and labor costs, and conservation of soil moisture (CTIC, 2011).

Maintaining optimum crop nutrition is critical in achieving high yields and quality in cotton. Pre-season soil test results for nitrogen, phosphorus, and potassium plus determination of pH, together with previous cropping and fertilization history determine the fertilizer and liming needs for the upcoming cotton crop. In the Southwest and West regions, monitoring soil salinity is of additional importance because cotton is most

sensitive to sodium and salts during the germination and seedling growth stage (Hake et al., 1996d). Soil salinity will severely delay emergence, which can make the plants more vulnerable to seedling disease.

Yield potential has generally been the most important factor considered by growers in variety selection (Smith and Cothren, 1999). Growers also need to consider fiber properties (*e.g.,* length, strength, micronaire, etc.), cold tolerance, seedling vigor, heat tolerance, leaf hairiness, insect and disease resistance, maturity, and a number of other factors. Cotton varieties are classified into three maturity groups: short-, medium-, or long-season varieties (Smith and Cothren, 1999). More determinate plants are planted in the short season northern portions of the cottonbelt and longer-season or more indeterminate varieties planted in the south. Growers in areas of western Texas and Oklahoma have tended to select 'stripper' or 'stormproof' varieties which produce a boll that is more resistant against yield loss under storm and hail conditions. "Picker" cotton varieties grown in the high plains of Texas produce large open bolls and are susceptible to yield loss from seasonally strong thunderstorm activity. Growers are advised to plant three or four varieties to reduce the risk of planting the entire farm to a poor-yielding variety or using traits that do not add value to their cropping system (NCCA, 2007).

VIII.C.2. Planting and Early Season

The yield potential of a cotton crop is determined in the first 30 to 40 days after seed is placed in the ground ([redacted] and [redacted], 1982). Planting date management is an important element in achieving early fruit set, and establishing a strong yield potential (Smith and Cothren, 1999). Cotton should be planted into prepared seedbeds that are firm, warm, and moist. Cotton specialists recommend planting cotton when soil temperatures at seeding depth are at 64° F or higher at 8 a.m. for three consecutive days, with a favorable five-day forecast ([redacted] and [redacted], 1982; Smith and Cothren, 1999). Under favorable conditions, emergence can occur anywhere from 5 to 15 days following planting. Once emerged, the cotton plant goes through a period of slow growth and development before entering phases of rapid vegetative and reproductive growth. The growth of the cotton plant is temperature dependent and growth ceases when the average daily temperature falls below 60° F (Hake et al., 1996b). It is during this early period of slow development that cotton must be protected from damaging weed, insect, and disease pests to prevent yield losses (Hake et al., 1996b; Smith and Cothren, 1999).

When planting is delayed significantly due to time constraints or weather conditions, growers are advised to switch to more determinate (short-season) type varieties. Planting good quality seed with a germination of 85% or higher is also important for establishing a good and uniform stand of cotton ([redacted] and [redacted], 1982). The single most important practice for minimizing damage from seedling diseases is selection of high-quality planting seed (Smith and Cothren, 1999). Most cottonseed sold commercially is treated with a fungicide to protect the germinating seed and seedlings from seed- and soil-borne pathogens (Smith and Cothren, 1999).

Plant population management contributes toward early fruiting, good fruit retention, and improved earliness of crop maturity (Smith and Cothren, 1999). Seeding rates vary

across the cotton growing region of the U.S. The seeding range will vary depending upon row-spacing, soil classification, available moisture, and overall environmental conditions. Cotton has the ability to compensate in response to row spacing and plant populations. However, higher plant densities tend to cause cotton plants to grow taller, develop more vegetative growth, and create more shading within the canopy (Smith and Cothren, 1999). These characteristics can result in delayed fruiting, alter the reproductive/vegetative balance, and decrease fruit retention. Conversely, low populations can delay overall plant maturity, allow sunlight to penetration through the canopy contributing to more weeds, and result in insufficient structure to produce adequate fruit which can influence overall harvestable yields.

VIII.C.3. Mid- to Late-Season

After early development, the next critical stage in the development of a cotton crop is rapid vegetative growth that includes the initiation of the first 'squares.' These floral buds develop into the subsequent fruiting forms called bolls. Fruiting development generally begins with the formation of fruiting branches on nodes four through eight ([redacted] and [redacted], 1982). After the accumulation of 40 to 60 days following emergence, the first square becomes visible, which is normally five to eight weeks after planting, depending on the area and temperature ([redacted] and [redacted], 1982; Hake et al., 1996c). Approximately 85% of the total bolls that are harvested come from squares set during the first four to five weeks of squaring ([redacted] and [redacted], 1982). Therefore, it is critical to properly manage cotton during this period to maximize yields.

Management practices, such as water management, plant nutrition management, and weed, disease, and insect control are critical during this reproductive growth phase. To maximize yield fruiting square and resulting boll retention is critical, especially the first bolls set on the plant. The first three 'positions' on each reproductive branch are the key sites for fruiting and will account for the vast majority of the plant's yield ([redacted] and [redacted], 1982). Further, the first-position, or squares nearest the main stem, will account for over 50% of the total lint produced per plant. The second-position squares account for another one-third or more of the harvest, while squares further out on each reproductive branch produce 15% or less of the final number of mature bolls harvested that contribute to yield ([redacted] and [redacted], 1982).

Most growers or crop consultants currently use a number of measurements during midseason to monitor and manage cotton plant growth. Although each will not be discussed in detail here, the grower may monitor any one or more of the following parameters: 1) plant height; 2) number of mainstem nodes; 3) node number of first fruiting branch; 4) total number of fruiting branches; 5) height-to-node ratios; and 6) square or fruit retention (Hake et al., 1996c). These parameters are commonly referred to as plant mapping. In general, as cotton is a perennial grown as an annual the cotton grower is seeking to favor reproductive growth at the expense of vegetative growth. This transition from vegetative to reproductive growth influences crop maturity and season length. Available options to influence cotton plant growth include the use of a plant growth regulator such as mepiquat chloride, fertility management (primarily nitrogen and potassium), and water management. Also, weed management and insect pest control are

important at mid-season, both of which can dramatically decrease both square and immature boll retention.

As the end of the growing season approaches, the yield is established and management efforts shift to protecting the crop yield and quality. The stage in cotton when vegetative growth ceases is generally referred to as "cut-out" (Hake et al., 1996a). When the nodes above the first-position white flower decline to four or five, cut-out has been reached. This is also the point at which the last effective bloom, which could contribute to yield, is on the plant. The timing of cut-out is critical for both yield and quality of cotton. If cut-out occurs too early, due to environment and management practices, the crop may not take full advantage of the available season. Late cut-out is often associated with poor early-season fruit retention and results in delayed maturity and harvest.

VIII.C.4. Preharvest and Harvest

The complete defoliation or desiccation of leaf tissue in preparation for harvest is a necessity with harvesting (Hake et al., 1996a). Leaves not only interfere with harvesting, but contribute to trash and moisture content, which influences ginning, cleaning, and overall quality of cotton lint. Effective defoliation is an essential step in the overall process of harvesting high quality cotton lint with the grower seeking to accomplish a complete, quick and efficient defoliation by chemical means. Defoliation attempts to speed up and control the natural process of senescence. An additional objective of defoliation is to kill or desiccate weeds that can reduce harvest efficiency, contribute to the weed seed bank, and reduce both the quality and value of the lint because of staining by vegetation (University of Georgia, 2012). Successful defoliation in cotton depends on a number of factors including: 1) plant-water status; 2) nitrogen fertility status; 3) weather conditions; and 4) the chemical defoliant(s) (Smith and Cothren, 1999). Defoliation should begin as early as possible by balancing the realistic yield potential of the crop with the need to preserve quality, schedule harvesting equipment, and minimize losses from weathering. Defoliation normally occurs when approximately 60% of the bolls are open (Smith and Cothren, 1999). Cotton is then harvested mechanically using pickers or strippers.

VIII.D. Management of Insect and Other Pests

Insect and mite pests are a common and continuous threat to cotton production in all regions of the U.S., leading to decreased yield and quality. Generally, fewer than 25 insect pests are considered persistent problems causing economic losses in cotton (Smith and Cothren, 1999). The susceptibility of cotton plants to insect pests varies across and within the various production regions. Insect and mite pests affect cotton production by decreasing yield and reducing quality. Nearly every phenological stage of cotton is susceptible to injury by one or more insect pests during the growing season. Therefore, cotton fields must be monitored regularly to detect the presence of insect pests. The susceptibility of cotton plants to economic yield losses from insect pests is influenced by pest population density, timing of infestations as related to plant phenology, local environmental conditions, and agronomic practices (Smith and Cothren, 1999).

Numerous insect species are observed in cotton fields across the U.S., but only a few are considered of economic importance. Yield loss and treatment costs for the most common insect pests in cotton in 2010 are shown in Table VIII-3. These data are estimates collected from surveys of county agents, extension specialists, private consultants, and research entomologists. Insect damage resulted in yield losses of approximately 986 thousand bales of cotton in 2010 or a 3.9% yield loss which represented an average loss of $22.56 per acre. The lepidopteran pests, bollworm/budworm, caused the greatest yield reductions followed by stink bugs and lygus insects, both of which are piercing and sucking insects. Thrips infested more acres in 2010 than any other insect in cotton. However, this insect ranked sixth in yield reductions, due to the damage occurring early in the growing season before the development of fruiting structures.

Successful and economical management of insect pests in cotton is accomplished through an integrated pest management approach of variety selection and implementation of cultural, biological, and chemical strategies (University of Georgia, 2011). Preplant tillage and crop rotation are important agronomic or cultural practices utilized to reduce insect populations prior to planting cotton. Other agronomic practices are utilized to promote early maturity and reduce that period of time the crop is susceptible to insect and mite pests, and to increase the probability that an acceptable yield can be produced before insect pest densities exceed economic threshold levels (Smith and Cothren, 1999).

Nematodes are another serious pest in cotton and have the potential to cause significant loss of yield, reduction in fiber quality, and crop maturity. Yield losses in cotton from nematodes exceed $400 million annually in the U.S (NCCA, 2007). Management decisions for controlling nematodes must be made prior to or at planting since few control options are available during the season.

Table VIII-3. Insect Losses in Cotton in U.S. in 2010[1]

Insect Pest	% Yield Reduction	Cotton Acres Infested	Cotton Acres Treated	Treatment Cost ($/Acre)	Cotton Bales Lost
Bollworm/Budworm	1.186	8,148,844	2,113,842	2.54	263,902
Stink bugs	0.724	6,712,988	2,782,462	3.45	162,397
Lygus spp.	0.677	5,932,835	2,458,413	6.86	191,826
Cotton Fleahopper	0.362	4,487,032	2,357,727	2.79	81,048
Aphids	0.286	7,133,029	1,270,253	1.39	60,377
Thrips	0.200	10,165,601	3,469,195	2.32	45,964
Spider mites	0.199	3,522,479	885,684	1.75	57,189
Fall Armyworm	0.199	2,762,701	203,109	0.20	41,256
Clouded Plant Bugs	0.024	614,569	213,683	0.14	6,465
Silverleaf Whitefly	0.020	508,430	111,902	0.81	4,935
Cutworms	0.003	487,946	543,570	0.19	699
Grasshoppers	0.001	1,293,128	53,300	0.02	373
Beet Armyworm	0.001	967,552	52,222	0.06	146
Loopers	0.001	735,998	9,160	0.01	197
Saltmarsh caterpillar	0.001	689,127	3,400	0.00	140
Banded winged whitefly	0.000	483,273	-	0.00	0
Southern Armyworms	0.000	242,500	-	0.00	0
Boll Weevil	0.000	115,470	25,920	0.01	0
Pink Bollworm	0.000	97,725	-	0.00	0
Cotton Leaf Perforator	0.000	14,988	14,988	0.00	0
European cornborer	0.000	0	-	0.00	0
Other Insects (1-4)	0.023	649,594	26,648	0.01	68,906
Total	**3.906**			**22.56**	**985,821**

[1](Williams, 2010).

VIII.E. Management of Diseases

Disease management is essential in cotton production to achieve optimum yields and economic returns. Plant pathologists estimate that diseases cause annual losses in cotton production of 1.8 million bales or a yield reduction of approximately 9.0 % in the U.S. (Blasingame et al., 2008). Seedling diseases, fungal wilts, root rots, and foliar diseases constitute the major disease complex in cotton (Smith and Cothren, 1999). Yield losses are often underestimated because most of the diseases are caused by soil-borne pathogens that attack the roots and cause little reduction of the plant size or change to the crop canopy (Smith and Cothren, 1999). These types of infestation can result in yield losses of as much as 20% without any awareness of the root infections by soil-borne pathogens.

The major seedling disease complex (*i.e.*, Pythium spp., Rhizoctonia spp., Fusarium spp., and Thielaviopsis spp.) are caused by fungal pathogens and are generally classified as seed-borne pathogens that occur on or in seed prior to planting and soil-borne pathogens that reside in soil (Smith and Cothren, 1999). The soil-borne pathogens are the most important causes of seedling disease and the most difficult to control. *Verticillium* wilt and *Fusarium* wilt are the two major fungal wilt diseases causing losses in cotton production. The pathogens penetrate root tips and enter the xylem vessels of the cotton plant and the plants subsequently develop the characteristics of wilt symptoms. Phymatotrichum root rot, macrophomina root rot, agrobacterium root rot and root gall are the primary diseases attacking roots of older cotton plants (Smith and Cothren, 1999). The foliar diseases are caused by pathogens that infect leaves, stems, bolls, and occasionally seedling roots. Bacterial blight, boll rot, fungal leaf spots, fungal boll rots, viran and mycoplasmal make up the primary foliar diseases.

An integrated management system is the best means of controlling diseases in cotton. This includes agronomic and cultural practices (*i.e.,* fertility, water management, crop rotation), use of resistant varieties, applications of fungicides and bactericides, and applications of biocontrol agents (Smith and Cothren, 1999). The single most important practice for minimizing damage from seedling diseases is selection of high-quality planting seed that has minimal seed coat damage and has been assessed for germination and vigor (Smith and Cothren, 1999). Seedbed conditions that encourage rapid germination and emergence will minimize seedling disease losses (NCCA, 2007). Selection of varieties with satisfactory levels of resistance is also an important step in the control of certain other diseases. Various cultural practices such as crop rotation, proper fertility and water management, clean tillage systems, early planting, eliminating weeds which are host plants to the pathogen, and practices that increase decomposition of crop residues can reduce the severity of diseases (Smith and Cothren, 1999). Fungicides are used to protect seeds and seedlings from seed- and soil-borne pathogens during their first few weeks of growth. Commercial cottonseed is normally treated and planted with a mixture of chemical fungicides applied to control these soil borne pathogens (Smith and Cothren, 1999). Fungicides are also used to prevent epidemics of foliar diseases when they approach economically damaging levels. An average of three fungicide treatments were made to cotton in 2007 (USDA-ERS, 2012a). Foliar fungicides are applied to approximately 2% of the cotton acreage (USDA-NASS, 2008).

VIII.F. Weed Management

Weed control in cotton is essential to maximize both cotton fiber yield and quality. In contrast to other crops, including corn and soybean, cotton emergence and above ground growth is relatively slow during the first few weeks after planting. The slow early growth of cotton does not permit the crop to aggressively compete against weed species that often grow more rapidly (Smith and Cothren, 1999). This is especially true under cool weather or adverse growing conditions which often prevail after cotton is planted. The extent or degree to which weeds interfere with cotton growth and yield is dependent on the species, densities, duration, and environmental conditions. For example, a single common cocklebur over 30 row-feet can reduce cotton yields by 8.85%, while a single prickly sida plant at the same density reduces cotton yields by only 0.26% (Smith and Cothren, 1999). Weed-crop competition studies have demonstrated that the control of weeds during the first four to eight weeks after cotton planting is critical as weeds compete against the crop for water, nutrients, light, and other resources necessary for growth (Smith and Cothren, 1999). Although late-season infestations may not impact yield, they reduce harvesting efficiency, contribute to the weed seed bank, and lower the lint grade (Vargas et al., 1996). Weeds can also have an impact on cotton diseases and insect management because certain weed species can be a host for *Rhizoctonia* and *Verticillium* wilt and harbor insects such as lygus bugs.

The occurrence and frequency of individual weed species in cotton vary greatly between and within each state and geographical growing region. Cultural and chemical control practices can cause shifts in the composition of weed populations (Smith and Cothren, 1999). Weed populations are affected over time by edaphic (soil-rated) factors, reproductive ability, control methods, cropping sequences, herbicide regimes, herbicide-resistance, climatic changes, and other environmental situations (Smith and Cothren, 1999). The most common weeds in cotton are not necessarily the most troublesome weeds. The degree of importance depends on the interference to cotton growth and yield, reduction in lint quality, and expense of control (Smith and Cothren, 1999). The proper identification of the weed species, especially in the immature stages of growth is essential to the development of an effective weed management program. Table 4 lists the common and species names of all weeds referred to in this petition. Tables VIII-5 through VIII-8 provide summaries of the most common weeds in cotton for each of the four major cotton growing regions (Southeast, Midsouth, Southwest, and West). Barnyardgrass, crabgrass, pigweed spp. (including Palmer amaranth), morningglory spp., common cocklebur, and common lambsquarters are common annual weed species in almost all cotton growing regions. Johnsongrass, bermudagrass and nutsedge are common perennial weed species. Weed species of the Solanaceae family, such as the nightshade spp. and groundcherry, are more common in the Southwest and West regions. Palmer amaranth, morningglory spp., and nutsedge spp. are not only common in cotton, but are frequently reported as some of the most troublesome or serious weed species in cotton (Webster et al., 2009).

Table VIII-4. Common and Scientific Names of Weeds Referred to in this Petition

Common Name	Scientific Name	Common Name	Scientific Name
Annual bluegrass	*Poa annua*	Little barley	*Hordeum pusillum*
Barnyardgrass	*Echinochloa crus-galli*	Jimsonweed	*Datura stramonium*
Bermudagrass	*Cynodon dactylon*	Johnsongrass	*Sorghum halepense*
Bindweed, field	*Convolvulus arvensis*	Junglerice	*Echinochloa colona*
Black nightshade (Eastern)	*Solanum ptychanthum*	Large Crabgrass	*Digitaria sanguinalis*
Broadleaf signalgrass	*Urochloa platyphylla*	Morningglory spp	*Ipomoea spp.*
Browntop millet	*Urochloa ramosa*	Mustard spp.	*Brassica spp.*
Buttercup	*Ranunculus spp.*	Nightshade, hairy	*Solanum physalifolium*
Carolina geranium	*Geranium carolinianum*	Nightshade, silverleaf	*Solanum elaeagnifolium*
Chickweed	*Stellaria media*	Nutsedge spp.	*Cyperus spp.*
Citronmelon	*Citrullus lanatus*	Palmer amaranth	*Amaranthus palmeri*
Common cocklebur	*Xanthium strumarium*	Pigweed spp.	*Amaranthus spp*
Common hempnettle	*Galeopsis tetrahit*	Prickly lettuce	*Lactuca serriola*
Common lambsquarters	*Chenopodium album*	Prickly sida	*Sida spinosa*
Common ragweed	*Ambrosia artemisiifolia*	Purple nutsedge	*Cyperus rotundus*
Crabgrass spp.	*Digitaria spp.*	Purslane, common	*Portulaca oleracea*
Crowfootgrass	*Dactyloctenium aegyptium*	Purslane, horse	*Trianthema portulacastrum*
Cupgrass, southwestern	*Eriochloa acuminata*	Red rice	*Oryza punctata*
Curly dock	*Rumex crispus*	Redweed	*Melochia corchorifolia*
Cutleaf evening-primrose	*Oenothera laciniata*	Russian thistle	*Salsola tragus*
Devil's claw	*Proboscidea louisianica*	Sandbur	*Cenchrus spp.*
Fall panicum	*Panicum dichotomiflorum*	Shepard's purse	*Capsella bursa-pastoris*
Florida beggarweed	*Desmodium tortuosum*	Sicklepod	*Senna obtusifolia*
Florida pusley	*Richardia scabra*	Smartweed spp.	*Polygonum spp.*
Foxtail spp.	*Setaria faberi*	Smellmellon	*Cucumis melo*
Giant foxtail	*Setaria spp.*	Sprangletop, red	*Leptochloa panicea*
Giant ragweed	*Ambrosia trifida*	Spreading dayflower	*Commelina diffusa*
Goosegrass	*Eleusine indica*	Spurge spp.	*Euphorbia spp.*
Groundcherry spp.	*Physalis spp.*	Spurred anoda	*Anoda cristata*
Henbit	*Lamium amplexicaule*	Sunflower	*Helianthus annuus*
Hemp sesbania	*Sesbania herbacea*	Texas blueweed	*Helianthus ciliaris*
Horseweed (marestail)	*Conyza canadensis*	Texas millet	*Urochloa texana*
Italian ryegrass	*Lolium multiflorum*	Texas panicum	*Urochloa reptans*
Kochia	*Kochia scoparia*	Tropical spiderwort	*Tradescantia ohiensis*
		Velvetleaf	*Abutilon theophrasti*

Table VIII-4. Common and Scientific Names of Weeds Referred to in this Petition (continued)

Common Name	Scientific Name
Virginia pepperweed	*Lepidium virginicum*
Volunteer corn	*Zea Mays*
Volunteer peanut	*Arachis hypogaea*
Common waterhemp	*Amaranthus rudis*
Tall waterhemp	*Amaranthus tuberculatus*
Wild lettuce	*Lactuca canadensis*
Wild mustard	*Sinapis arvensis*
Wild radish	*Raphanus raphanistrum*
Woolyleaf bursage	*Ambrosia grayi*
Yellow nutsedge	*Cyperus esculentus*

Table VIII-5. Common Weeds in Cotton Production in the Southeast Region of the U.S.[1,2]

Crabgrass spp.(6)	Pigweed spp.(3)	Crowfootgrass(1)
Morningglory spp.(6)	Common cocklebur(2)	Horseweed (marestail)(1)
Prickly sida(5)	Common lambsquarters(2)	Jimsonweed(1)
Florida pusley(4)	Common ragweed(2)	Johnsongrass(1)
Nutsedge spp.(4)	Florida beggarweed(2)	Smartweed spp.(1)
Sicklepod(4)	Palmer amaranth(2)	Spurge spp.(1)
Broadleaf signalgrass(3)	Texas millet(2)	Volunteer peanut(1)
Goosegrass(3)	Bermudagrass(1)	

[1]OK data (Webster et al., 2009).
[2]Number provided in parenthesis is the number of states out of the six total states (AL, FL, GA, NC, SC, and VA) in the Southeast region reporting each weed as one of the ten most common weeds.

Table VIII-6. Common Weeds in Cotton Production in the Midsouth Region of the U.S.[1,2]

Morningglory spp.(5)	Velvetleaf(3)	Common cocklebur(1)
Broadleaf signalgrass(4)	Barnyardgrass(2)	Cutleaf evening-primrose(1)
Crabgrass spp.(4)	Horseweed (marestail)(2)	Goosegrass(1)
Nutsedge spp.(4)	Johnsongrass(2)	Hemp sesbania(1)
Prickly sida(4)	Palmer amaranth(2)	Henbit(1)
Spurge spp.(4)	Bermudagrass(1)	Spurred anoda(1)
Pigweed spp.(3)	Browntop millet(1)	

[1]AR, LA & MO data (Webster et al., 2009); MS & TN data (Webster et al., 2005).
[2]Number provided in parenthesis is the number of states out of the five total states (AR, LA, MS, MO, & TN) in the Midsouth region reporting each weed as one of the ten most common weeds.

Table VIII-7. Common Weeds in Cotton Production in the Southwest Region of the U.S.[1,2]

Johnsongrass(4)	Mustard spp.(2)	Shepard's purse(1)
Nutsedge spp.(4)	Pigweed spp.(2)	Smartweed(1)
Cocklebur, common(3)	Russian thistle(2)	Smellmelon(1)
Palmer amaranth(3)	Barnyardgrass(1)	Spurred anoda(1)
Silverleaf Nightshade (3)	Bermudagrass(1)	Sprangletop, red(1)
Common lambsquarters(2)	Bindweed, field (1)	Sunflower(1)
Large Crabgrass(2)	Foxtail spp.(1)	Texas blueweed(1)
Devil's claw(2)	Groundcherry spp.(1)	Texas millet(2)
Morningglory spp.(2)	Kochia(1)	Velvetleaf(1)
	Horseweed (marestail)(1)	Woolyleaf bursage(1)

[1]OK data (Webster et al., 2009); KS – [redacted] Kansas State University – Personal Communication November, 2010; NM – [redacted] New Mexico State University – Personal Communication November, 2010; TX – [redacted] Texas A&M University - Personal Communications November, 2010.
[2]Number provided in parenthesis is the number of states out of the four total states (OK, KS, TX, & NM) in the Southwest region reporting each weed as one of the ten most common weeds.

Table VIII-8. Common Weeds in Cotton Production in the West Region of the U.S.[1,2]

Barnyardgrass(2)	Groundcherry spp.(1)	Nightshade, black(1)
Morningglory spp.(2)	Lambsquarters, common(1)	Nightshade, hairy(1)
Sprangletop(2)	Johnsongrass(1)	Nightshade, silverleaf(1)
Bermudagrass(1)	Junglerice(1)	Palmer amaranth(1)
Bindweed, field(1)	Nutsedge spp.(1)	Purslane, common(1)
Cupgrass, southwestern(1)	Pigweed spp.(1)	Purslane, horse(1)
		Volunteer corn(1)

[1]Source:AZ – [redacted] University of Arizona – Personal Communication, November, 2010; CA – [redacted] University of California - Personal Communication November, 2010.
[2]Number provided in parenthesis is the number of states out of the two total states (AZ & CA) in the West Region reporting each weed as one of the ten most common weeds.

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

Weeds in cotton are controlled through the integrated use of various cultural, mechanical, and chemical methods (Hake et al., 1996d). Crop rotation, or the lack of rotation, in conjunction with other weed control methods, can play an important factor on the weed spectrum and drastically impact weed populations (Smith and Cothren, 1999). Historically, mechanical tillage and hand hoeing were the most important tools in cotton weed control. Current weed management practices include as many as five tillage operations in conventional tillage systems and two or three tillage operations in mulch tillage, or no-till systems (USDA-ERS, 2012b). Approximately, 38% of the total cotton acres are post-plant cultivated and within conventional tillage systems, over 50% cotton acres are cultivated for weed control (USDA-ERS, 2012b).

The use of chemical methods for weed control began to develop in cotton in the 1940s and 1950s with the discovery and development of several selective herbicides (Buchanan, 1992). Dinoseb, chloropropham, dalapon, and diuron were developed and used in cotton. Despite the increased use of herbicides in the late 1950s, less than 10% of the total U.S. cotton acreage received a herbicide treatment. However, herbicide use rapidly accelerated in the 1960s as a series of more selective herbicides were introduced into the market. These herbicides provided good weed control with less cotton injury than most products used a decade earlier. These products included trifluralin, DSMA/MSMA, prometryn, and fluometuron. These herbicides, representing different chemical families and modes-of-action, are still widely used today. Additional herbicides were introduced during the 1970s that were efficient, effective, and relatively economical on a wide range of weed species. Glyphosate was introduced in the early 1970s and quickly became one of the most effective herbicides for nonselective spot treatments for control of johnsongrass and other weeds (Buchanan, 1992). Glyphosate was also an effective burndown treatment within no-till cotton production. The use of dinoseb for broadleaf weed control was halted in 1987 with the suspension of the registration by the Environmental Protection Agency (McWhorter and Bryson, 1992). Registrations were also discontinued for dinitramine, flurachloralin, profluralin, dalapon, dipropetryn, and

perfluidone. Numerous additional selective herbicides for grass and broadleaf weed control were introduced in the 1980s in cotton including fluazifop, metolachlor, oxyfluorfen, and sethoxydim. However, the use of these products does not equal the acreage treated with the herbicides which were discontinued (Buchanan, 1992). By the mid 1980s, there were 33 herbicides and herbicide combinations applied in cotton (Buchanan, 1992). The greatest use of herbicides on a per-acre basis was in the Midsouth which averaged 5.7 herbicide applications per acre each year. During the 1990s, the herbicides lactofen, bromoxynil, clethodim, clomazone, quizalifop, and pyrithiobac were introduced for use in cotton.

The first biotechnology-derived herbicide-tolerant cotton became available in 1995 and provided tolerance to bromoxynil (Stalker et al., 1996). Approximately 50,000 acres of bromoxynil-tolerant cotton were planted the year of introduction and approximately 2500 growers planted 200,000 acres of bromoxynil-tolerant cotton in 1996 (Smith and Cothren, 1999). The second herbicide-tolerant cotton product, the first generation glyphosate-tolerant cotton, was introduced in 1997. Glyphosate-tolerant cotton in combination with glyphosate herbicide became the standard program for weed management in cotton. The first generation glyphosate-tolerant weed control system in cotton provided postemergence control of a broad spectrum of weeds with excellent early-season crop safety (Wilcut et al., 2003). Glyphosate-tolerant cotton expanded the grower's options for weed management and made the mechanics of weed control much easier, more convenient, and less expensive (Carpenter and Gianessi, 2001; Wilcut et al., 2003). This system also provided a better fit into no-till and reduced-tillage systems, resulting in an increase in conservation tillage systems in cotton (Baldwin and Baldwin, 2002; Carpenter and Gianessi, 2001). Glyphosate could be applied postemergence to glyphosate-tolerant cotton from emergence through the four-leaf stage. After the four leaf stage and up to layby (canopy closure in the row), glyphosate had to be applied as a post-directed spray between the crop rows to minimize contact with the cotton plants to prevent potential crop injury.

In 2003, glyphosate-tolerant cotton was planted on approximately 59% of the cotton acres in the U.S. (USDA-NASS, 2003). Glyphosate was the most widely used herbicide in cotton in terms acres treated (USDA-NASS, 2004). However, cotton growers continued to use a variety of herbicides with various modes-of-action in glyphosate-tolerant cotton. Trifluralin and pendimethalin were used on nearly half of the U.S. cotton acreage for small seeded grass and broadleaf weed control. Various substituted urea herbicides (diuron, prometryn, fluometuron and linuron) were also used on 50% of the U.S. cotton acreage (USDA-NASS, 2004). The soil residual activity of these herbicides on a number of weed species provided additional season-long control of continuously germinating weeds in glyphosate-tolerant cotton systems (Askew et al., 2002; Wilcut et al., 2003). Other herbicide products representing additional modes-of-action, including carfentrazone, MSMA, pyrithiobac and metolachlor, were also used on cotton ranging from four to 11% of the acres (USDA-NASS, 2002).

In 2006, a second generation glyphosate-tolerant product was introduced providing increased tolerance to glyphosate in the reproductive stages of cotton. This allowed for an expanded window for over-the-top applications of glyphosate in cotton. Glyphosate

can be applied over-the top in second generation glyphosate-tolerant cotton from emergence up to 7 days prior to harvest. With this additional application flexibility, growers were able to more effectively manage weeds in cotton using over-the-top applications as opposed to post-directed or hooded sprayer applications with previous glyphosate-tolerant varieties. In addition, foliar insecticides could be combined with glyphosate in a single application during the season for secondary pests such as thrips, aphids, and plant bugs. Mepiquat chloride, a plant growth regulator commonly used in cotton production to reduce vegetative growth and increase fruit retention, could also be applied with glyphosate in a single application. In 2010, approximately 78% of the cotton acreage was planted to herbicide-tolerant cotton, which was nearly all glyphosate-tolerant (Brookes and Barfoot, 2012).

The third herbicide-tolerant cotton product, glufosinate-tolerant cotton, was introduced in 2003. Only 3% was planted to glufosinate-tolerant cotton in 2010 (USDA-ERS-FAS, 2010). Approximately 50% of the acres planted to cotton varieties containing both herbicide- and insect-tolerant traits (USDA-NASS, 2011a).

Table VIII-9 provides a summary of the herbicide applications registered for use in cotton in 2010, the data are discussed below. Herbicides are used on essentially all (99+%) cotton acres in the U.S. (Monsanto Company, 2011). A total of 32.8 million pounds of herbicide active ingredient were applied in cotton in 2010. Glyphosate was the predominate herbicide used in cotton with 19.6 million pounds active ingredient being applied on 91% of the acres. The number of glyphosate applications, on glyphosate-tolerant cotton, average approximately 2.4 applications per year at an average rate of 2.0 pounds of glyphosate active ingredient per acre per crop year (Monsanto, 2011). Dinitroanaline herbicides (pendimethalin and trifluralin) were applied on 53% of the cotton acres. Diuron (18%), flumioxazin (16%), metolachlor (16%), pyrithiobac (15%), fomesafen (13%), and 2,4-D (13%) were also frequently used herbicides in cotton (Monsanto Company, 2011).

According to USDA-ERS (2012a) statistics, growers make on average a total of four herbicide applications in cotton during the growing season. Approximately 16-19% of the growers utilizing the latest glyphosate-tolerant cotton varieties applied a fall herbicide application to control weeds prior to planting cotton depending on their crop rotation (Prince et al., 2011). Approximately 53-97% of the growers applied spring burndown treatments in glyphosate-tolerant cotton, which consisted of predominately glyphosate and/or synthetic auxins (2,4-D, dicamba).

Dicamba is currently labeled for use in cotton, although dicamba use is limited because applications are restricted to early preplant only, due to cotton injury. Before planting cotton, a minimum accumulation of one inch of rainfall or overhead irrigation must occur and a waiting interval of 21 days is required per 0.25 lbs acid equivalent (a.e.) or less. Dicamba-treated acres have increased in cotton primarily because it is a leading herbicide recommendation for glyphosate-resistant marestail (horseweed) in the Midsouth region ([redacted] et al., 2006).

Glufosinate may be used for weed control in non-glufosinate-tolerant cotton when applied with a hood sprayer in-crop to avoid contact with cotton plants. Glufosinate can also be applied in glufosinate-tolerant cotton from emergence up to the early bloom growth stage.

Approximately 15, 39, and 42% of growers made 1, 2, and 3 in-crop applications of glyphosate in continuous cotton, respectively (Prince et al., 2011). Although glyphosate is used extensively in glyphosate-tolerant cotton, non-glyphosate herbicides with different modes-of-action are also utilized to provide residual weed control, improve the control of certain weed species, extend weed control, and/or control resistant weeds. The use of herbicides with different modes-of-action is an effective practice to reduce the potential risk of weeds developing resistance to glyphosate or other herbicide modes-of-action. Approximately 49-76% of growers applied non-glyphosate herbicides prior to planting, at planting, or postemergence in glyphosate-tolerant cotton in 2010 depending on cropping system (Prince et al., 2011). The non-glyphosate herbicides were ALS inhibitors (trifloxysulfuron, pyrithiobac), photosystem II inhibitors (prometryn, fluometuron, diuron), mitosis inhibitors (metolachlor), PPO inhibitors (flumioxazin, fomesafen), and synthetic auxins (2,4-D, dicamba).

Weed management in conventional cotton varieties is very similar. The major difference in the herbicide programs is that alternative postemergence herbicides or herbicide tank mixtures are applied in place of glyphosate as in-crop post applications. Glyphosate can still be applied alone or in combinations with other herbicides in preplant burndown or preharvest applications in conventional cotton. A herbicide or combination of herbicides (trifluralin, pendimethalin, fluometuron, fomesafen, flumioxazin) is generally applied at planting for residual grass and broadleaf weed control. Generally, at least two in-crop post applications are made for control of emerged weeds during the growing season. Pyrithiobac, trifloxysulfuron, prometryn, clethodim, and sethoxydim are some of the more common herbicides used post in cotton. In addition, a layby application of one or more herbicides is applied such as diuron, MSMA, prometryn, or trifloxysulfuron.

Tables VIII-10 through VIII-14 provide a summary of the control ratings of common weed species to various herbicides and herbicide combinations in cotton. These tables list only the most commonly used herbicides or herbicide treatments in cotton production and control ratings are for non-glyphosate-resistant weeds. Seldom would one field or farm have all weed species, but they generally have a mixture of grass and broadleaf weed species. These ratings are utilized to facilitate the selection of a herbicide program for the cotton crop, which offers the best overall control of the weed species. Dinitroanalines (trifluralin and pendimethalin) provide effective control of most listed annual grasses, but only certain broadleaf species. Postemergence treatments of quizalofop, fluazifop, sethoxydim and clethodim are effective on the annual grasses and perennial grasses listed such as johnsongrass and bermudagrass, but provide no control of the broadleaf species. On the other hand, preemergence or postemergence applications of fluometuron or pyrithiobac provide good control of many broadleaf weeds and poor or no control of most grasses. In-crop applications of glyphosate and glufosinate provide good to excellent control of a broad spectrum of annual grass and broadleaf weeds. However,

glyphosate provides more effective control of perennial weeds such as bermudagrass, johnsongrass, and nutsedge species as compared to glufosinate. In addition, glyphosate combinations are the most effective herbicide treatments for silverleaf nightshade, Texas blueweed, and woolyleaf bursage, which are problem weeds in the Southwest region. Post-directed layby applications of MSMA in combination with diuron, flumioxazin, or prometryn and the premix combination of prometryn/trifloxysulfuron provide broad spectrum weed control. Due to the broad range of weed species present in cotton, multiple treatments and/or combinations of herbicides are used to achieve effective season-long weed control in cotton.

Table VIII-9. Herbicide Applications Registered for Use in Cotton in 2010[1]

Herbicide	Chemical Family	Mode of Action (MOA)	Cotton Acres Treated (%)	Cotton Acres Treated per MOA (%)	Quantity Applied (1000 lbs a.i. [2])	Total Quantity Applied/MOA (1000 lbs a.i.[2])
Glyphosate	Glycine	EPSPS inhibitor	91	91	19,602	19,602
Pendimethalin	Dinitroanaline	Microtubule inhibitor	18	53	1,584	5,138
Trifluralin	Dinitroanaline		35		3,554	
Diuron	Urea	PSII inhibitor	18	34	1,527	2,827
Prometyrn	Triazine		8		650	
Fluometuron	Urea		7		619	
Linuron	Urea		<1		31	
Carfentrazone	Triazolinone	PPO inhibitor	1	30	2	465
Flumioxazin	N-phenylphthalimide		16		114	
Fomesafen	Diphenylether		13		342	
Lactofen	Diphenylether		<1		1	
Oxyfluorfen	Diphenylether		<1		6	
Pyraflufen	Phenylpyrazole		<1		<1	
2,4-D	Phenoxy	Synthetic Auxin	13	21	891	1,084
2,4-DB	Phenoxy		<1		1	
Dicamba	Benzoic acid		8		192	

Table VIII-9. Herbicide Applications Registered for Use in Cotton in 2010[1](continued)

Herbicide	Chemical Family	Mode of Action (MOA)	Percent of Cotton Acres Treated	Percent of Cotton Acres Treated per MOA	Quantity Applied (1000 lbs a.i.[2])	Total Quantity Applied/MOA (1000 lbs a.i.[2])
Pyrithiobac	Benzoate	ALS inhibitor	15	20	72	75
Thifensulfuron	Sulfonylurea		<1		<1	
Tribenuron	Sulfonylurea		<1		<1	
Trifloxysulfuron	Sulfonylurea		4		3	
Acetochlor	Chloroacetamide	Long-chain fatty acid inhibitor	<1	16	47	1,813
Metolachlor	Chloroacetamide		16		1,766	
Paraquat	Bipyridylium	Photosystem-I-electron diverter	10	10	547	547
Glufosinate-ammonium	Phosphinic acid	Glutamine synthesis inhibitor	8	8	535	535
MSMA	Organoarsenical	Cell membrane disruption	6	6	747	747
Clethodim	Cyclohexanedione	ACCase inhibitor	<1	1	6	7
Fluazifop	Aryloxyphenoxy-propionate		<1		1	
Diflufenzopyr	Semicarbazone	Auxin transport	<1	<1	<1	<1
Clomazone	Isoxazolidinone	Diterpene synthesis inhibitor	<1	<1	10	10
Total				99.4		32,856

[1](Monsanto, 2011).
[2]a.i.= active ingredient.

Table VIII-10. Grass Weed Species Control Ratings to Preplant Burndown Herbicides in Cotton

	Common Grass Weeds[1]											
Product	AB[2]	BG[2]	CG[2]	GFT[4]	GG[2]	IRG[2]	JGs[2]	LB[2]	RR[4]	SB[2]	TP[2]	VC[2]
2,4-D	N	N	N	0	N	N	N	N	0	N	N	N
Glufosinate	6[3]	-	8[3]	-	8[3]	8[3]	9[3]	7[3]	-	-	-	-
Glufosinate + 2,4-D or dicamba	6[4]	-	7[4]	8[4]	-	5[4]	-	7[4]	7[4]	-	-	-
Glyphosate	E	F	E	8	E	G	G-E	E	8	E	E	E
Glyphosate + 2,4-D	E	F	G-E	8	G-E	G	G	E	8	G-E	G-E	E
Glyphosate + dicamba	E	F	G-E	8	G-E	G	G	E	8	G-E	G-E	E
Glyphosate + carfentrazone or pyraflufen	E	F	E	-	E	G	G-E	E	-	E	E	E
Glyphosate + diuron	E	F	G	-	G	F	F-G	E	-	G	G	E
Glyphosate + thifensulfuron/ tribenuron	E	F	E	-	E	G	G-E	E	-	E	E	E
Glyphosate + flumioxazin	E	F	E	8	E	G	G-E	E	8	E	E	E
Paraquat	G-E	P	F-G	8	F-G	F	P	G	7	G	G	F-G
Paraquat + diuron	E	P	G	-	G	F-G	P	G-E	-	G	G-E	F-G

[1]Weed Species: AB = annual bluegrass, BG = bermudagrass, CG = crabgrass, GFT = giant foxtail, GG = goosegrass, IRG = Italian ryegrass, JGs = seedling johnsongrass, LB = little barley, RR = red rice, SB = sandbur, TP = Texas millet (Texas panicum), VC = volunteer corn.
[2](University of Georgia, 2012). Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.
[3] (MSU, 2012). Rating scale: 0-3 = none to slight, 4-6 = fair, 7-8 = good, 9-10 = excellent control.
[4] (University of Arkansas, 2011). Rating Scale: 0 = no control, 10 = 100% control.

Table VIII-11. Grass Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton

	Common Grass Weeds & Nutsedge[1]														
Product	**BYG[2]**	**BSG[3]**	**CG[3]**	**CFG[3]**	**CPG[4]**	**FT[3]**	**GG[3]**	**JR[5]**	**JGs[3]**	**ST[4]**	**TP[3]**	**BG[3]**	**JGr[3]**	**NSy[3]**	**NSp[3]**
Preplant Incorporated Only															
Pendimethalin	9	G	E	E	C	E	E	C	E	-	G	N	P	N	N
Trifluralin	9	G	E	E	C	E	E	C	E	C	G	N	P	N	N
Preemergence															
Pendimethalin	-	F	G	G	C	G	G	C	G	-	F	N	P	N	N
Clomazone	-	E	E	G	-	E	E	-	G	-	F	P-F	N	N	N
Fluometuron	7	P	F-G	F-G	-	F-G	F	-	P	-	P	N	N	N	N
Diuron	7	P	F-G	F-G	C	-	F	C	P	N	P	N	N	N	N
Fomesafen	-	F-G	F-G	-	-	-	-	-	-	-	F	N	-	G-E	-
Pyrithiobac	6	P	P	-	-	P	P-F	-	F-G	-	N	N	N	F	F
Postemergence Residual Control															
Metolachlor	-	F-G	E	E	C	E	E	C	F	C	P-F	N	P	F	P
Pyrithiobac	-	P	P	-	-	P	P-F	-	F	-	N	N	N	P-F	F
Trifloxsulfuron	-	P	P	P	-	P	P	-	P	-	P	N	N	-	-

Table VIII-11. Grass Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton (continued)

	Common Grass Weeds & Nutsedge[1]														
Product	**BYG[2]**	**BSG[3]**	**CG[3]**	**CFG[3]**	**CPG[4]**	**FT[3]**	**GG[3]**	**JR[4]**	**JGs[3]**	**ST[4]**	**TP[3]**	**BG[3]**	**JGr[3]**	**NSy[3]**	**NSp[3]**
Postemergence Over-The-Top															
Quizalofop	8	G	G	G	-	E	G	-	E	-	G	G	E	N	N
Fluazifop	7	G-E	G	F	C	E	G	C	G-E	C	G	G	G-E	N	N
Sethoxydim	8	E	G-E	F-G	C	E	G-E	C	G-E	C	E	F	G	N	N
Clethodim	8	E	G-E	G	C	E	G-E	C	E	C	E	G	G-E	N	N
MSMA	-	P	P	P	P	-	P	N	P	N	N-P	N	P	P	N-P
Fluometuron	-	P	P-F	P-F	-	-	P-F	-	P	-	N	N	N	N	N
Pyrithiobac	2	N	N	N	N	N-P	N-P	N	P	N	N	N	N-P	P-F	P-F
Trifloxsulfuron	7	N	P	N	N	N-P	N-P	N	F	N	N-P	N	P	G	F-G
Glyphosate	9	E	E	E	C	E	E	C	E	C	E	F	G-E	F	F-G
Glyphosate + Pyrithiobac	9	E	E	E	-	E	E	-	E	-	E	F	G-E	F-G	F-G
Glyphosate + Trifloxsulfuron	-	E	E	E	-	E	E	-	E	-	E	F	G-E	G-E	G
Glufosinate	8	G	G	G	C	G	P	C	G	C	G	N	F	P	P

Table VIII-11. Grass Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton (continued)

	Common Grass Weeds & Nutsedge[1]														
Product	**BYG[2]**	**BSG[3]**	**CG[3]**	**CFG[3]**	**CPG[4]**	**FT[3]**	**GG[3]**	**JR[4]**	**JGs[3]**	**ST[4]**	**TP[3]**	**BG[3]**	**JGr[3]**	**NSy[3]**	**NSp[3]**
Postemergence Directed – Layby															
MSMA	-	F	F	F	P	F	F	N	F	N	P	N	P	F-G	F
Diuron + MSMA	9	G	G	F-G	-	F-G	F-G	-	F-G	-	F	N	P	G	F
Prometryn + MSMA	9	F-G	F-G	F-G	-	F-G	F-G	-	F-G	-	F	N	P	F-G	F
Flumioxazin + MSMA	9	F	F	F	-	F	F	-	F	-	P-F	N	P	G	F-G
Prometryn/ trifloxysulfuron +MSMA	9	F-G	F-G	F-G	-	F-G	F-G	-	F-G	-	F	N	P	E	E

[1]Weed species: BYG = barnyardgrass, BSG = broadleaf signalgrass, CG = crabgrass, CFG = crowsfootgrass, CPG = cupgrass, FP = fall panicum, GG = goosegrass, JR = junglerice, JGs = seedling johnsongrass, ST = sprangletop, TP = Texas panicum (Texas millet), BG = bermudagrass, JGr = rhizome johnsongrass, NSy = yellow nutsedge, and NSp = purple nutsedge.
[2](University of Arkansas, 2011). Rating Scale: 0 = no control, 10 = 100% control.
[3](University of Georgia, 2012).Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.
[4] (University of California, 2012). Ratings Key: C = control, P = partial control, N = no control, - = no information.

Table VIII-12. Broadleaf Weed Species Control Ratings to Preplant Burndown Herbicides in Cotton

	Common Broadleaf Weeds[1]												
Product	**CD[2]**	**BC[3]**	**CG[3]**	**CW[3]**	**CM[3]**	**CLP[3]**	**HB[3]**	**MT[3]**	**PA[3]**	**SA[3]**	**VP[3]**	**WL[3]**	**WR[3]**
2,4-D	9	G	F	P	F	E	P-F	G-E	F	F-G	G-E	G	G
Glufosinate[4]	-	-	8	10	-	7	6	9	-	-	9	-	-
Glufosinate + 2,4-D or dicamba[2]	8	10	8	10	-	8	10	9	9		10	-	-
Glyphosate	7	G-E	P-F	E	G-E	P-F	G-E	G-E	E	G	G	G-E	F-G
Glyphosate + 2,4-D	9	E	F-G	E	E	E	E	E	E		E	G-E	E
Glyphosate + dicamba	9	E	G	E	E	G	E	E	E	-	G-E	G-E	G-E
Glyphosate + carfentrazone or pyraflufen	-	G-E	F-G	E	E	F	E	G-E	E	G	G	G-E	G
Glyphosate + diuron	-	G-E	G	E	G-E	F-G	E	G-E	E	G	G	G-E	G
Glyphosate + thifensulfuron / tribenuron nuron	-	G-E	G-E	E	G-E	F	E	G-E	E	-	G	G-E	E
Glyphosate + flumioxazin	7	G-E	G	E	E	F-G	E	G-E	E	-	G-E	E	G
Paraquat	5	E	G-E	E	F	F	G-E	P-F	F-G	F-G	G	P	F-G
Paraquat + diuron	-	E	E	E	G	G-E	E	F-G	G-E	F-G	G	F	G-E

[1]Weed Species: CD = curly dock, BC = buttercup, CG = Carolina geranium, CW = chickweed, CM = citronmelon, CLP = cutleaf primrose, HB = henbit, MT = marestail, PA = Palmer amaranth, SA spurred anoda, VP = Virginia pepperweed, WL = wild lettuce, WR = wild radish.
[2] (University of Arkansas, 2011). Rating Scale: 0 = no control, 10 = 100% control.
[3](University of Georgia, 2012). Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.
[4] (MSU, 2012). Rating scale: 0-3 = none to slight, 4-6 = fair, 7-8 = good, 9-10 = excellent control.

Table VIII-13. Broadleaf Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton - Part I

	Common Broadleaf Weeds[1]												
Product	**CB[2]**	**DC[3]**	**FB[2]**	**FP[2]**	**GC[4]**	**HS[2]**	**NS[4]**	**JW[2]**	**LQ[2]**	**MG[2]**	**PA[2]**	**PW[2]**	**PS[2]**
Preplant Incorporated													
Pendimethalin	N	N	P	E	N	N	N	N	G-E	P	G	G-E	N
Trifluralin	N	N	P	E	N	N	N	N	G-E	P	G	G-E	N
Preemergence													
Pendimethalin	N	N	P	F-G	N	N	N	N	G	P	P-F	F-G	N
Clomazone	F	F	F-G	F-G	-	F	-	G	G	P-F	N-P	P	E
Fluometuron	F-G	F-G	G-E	F-G	-	P	-	G	G-E	G	F	G-E	G
Diuron	F	F	G	P-F	C	P	C	G	G-E	F	F-G	G-E	F
Fomesafen	G	F-G	P	P	-	P	-	-	E	P-F	E	E	-
Pyrithiobac	N-P	F-G	G	F	-	P	-	F-G	G	F	G-E	E	G
Postemergence Residual Control													
Metolachlor	P	N	P-F	G	-	P	-	-	F	P	G	G-E	F
Pyrithiobac	N-P	G	G	F	-	P	-	F-G	G	F	G-E	G-E	G
Trifloxsulfuron	-	G	F-G	P-F	-	-	-	-	-	-	P-F	F	-

Table VIII-13. Broadleaf Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton – Part I (continued)

Product	Common Broadleaf Weeds[1]											
	CB[2]	DC[3]	FB[2]	FP[2]	GC[4]	HS[2]	NS[3]	JW[2]	LQ[2]	MG[2]	PA[2]	PW[2]	PS[2]
Postemergence Over-The-Top													
Quizalofop	N	N	N	N	-	N	-	N	N	N	N	N	N
Fluazifop	N	N	N	N	N	N	N	N	N	N	N	N	N
Sethoxydim	N	N	N	N	N	N	N	N	N	N	N	N	N
Clethodim	N	N	N	N	N	N	N	N	N	N	N	N	N
MSMA	E	P-F	E	N-P	P	-	P	P	P	P-F	P	P	P
Fluometuron	F-G	F-G	G	P-F	-	-	-	G	G	G	P-F	F	F-G
Pyrithiobac	G	G	G	N-P	P	G-E	C	E	N	G	F	G	F
Trifloxsulfuron	G-E	G	G-E	P	-	-	-	N	G	G	P-F	F-G	N
Glyphosate	E	E	E	P-G	C	P-F	C	E	G	F-G	E	E	F-G
Glyphosate + Pyrithiobac	E	E	E	P-G	-	G-E	-	E	G	G-E	E	E	G
Glyphosate + Trifloxsulfuron	E	E	E	P-G	-	-	-	E	E	E	E	E	G
Glufosinate	E	G-E	G	F	C	-	C	E	E	E	F-G	G	F
Postemergence Directed – Layby													
MSMA	E	P-F	E	P	P	N	P	F	P-F	F	P	P-F	P
Diuron + MSMA	E	G-E	E	F	-	P-F	-	G	G	G-E	G	G-E	G-E
Prometryn + MSMA	E	G-E	E	F	-	P-F	-	G	G	G-E	F	G	G-E
Flumioxazin + MSMA	E	G-E	E	F-G	-	-	-	E	F-G	E	F-G	G-E	G-E
Prometryn/ trifloxysulfuron + MSMA	E	G-E	E	F	-	-	-	G	G-E	E	G	G-E	G-E

[1]Weed species: CB = common cocklebur, DC = devil's claw, FB = Florida beggarweed, FP = Florida pusley, GC = ground cherry, HS = hemp sesbania, NS = nightshade, JW = jimsonweed, LG = Common lambsquarters, MG = morningglory species, PA = Palmer amaranth, PW = pigweed species, PS = Prickly sida.

[2](University of Georgia, 2012). Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control

[3]Personal communications with [redacted] Texas A & M University - 2011, Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.

[4] (University of California, 2012). Ratings Key: C = control, P = partial control, N = no control, - = no information.

Table VIII-14. Broadleaf Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton – Part II

	Common Broadleaf Weeds[1]										
Product	**PL[2]**	**RW[2]**	**RdW[2]**	**SP[2]**	**SG[2]**	**SN[3]**	**SW[2]**	**TB[3]**	**TSW[2]**	**VL[4]**	**WB[3]**
Preplant Incorporated											
Pendimethalin	E	N	N	N	N	N	N	N	N	0	N
Trifluralin	E	N	N	N	N	N	N	N	N	0	N
Preemergence											
Pendimethalin	G	N	N	N	N	N	N	N	N	0	N
Clomazone	G-E	G	G-E	P	N	N	E	N	F	10	N
Fluometuron	E	E	E	G	P-F	N	G	N	F	3	N
Diuron	E	G	G-E	F	F	N	G	N	P-F	7	N
Fomesafen	G	G	-	P	-	N	-	N	N	1	G
Pyrithiobac	G	N-P	G-E	P-F	G	N	G	N	P	8[5]	N
Postemergence Residual Control											
Metolachlor	G	P	-	P	P-F	N	-	N	E	-	N
Pyrithiobac	G	N-P	G-E	P	G	N	G	N	P	-	N
Trifloxsulfuron	-	-	-	P-F	-	N	-	N	-	-	N

Table VIII-14. Broadleaf Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton – Part II (continued)

	Common Broadleaf Weeds[1]										
Product	**PL[2]**	**RW[2]**	**RdW[2]**	**SP[2]**	**SG[2]**	**SN[3]**	**SW[2]**	**TB[3]**	**TSW[2]**	**VL[4]**	**WB[3]**
Postemergence Over-The-Top											
Quizalofop	N	N	N	N	N	N	N	N	N	0	N
Fluazifop	N	N	N	N	N	N	N	N	N	0	N
Sethoxydim	N	N	N	N	N	N	N	N	N	0	N
Clethodim	N	N	N	N	N	N	N	N	N	0	N
MSMA	P-F	P-F	N	P-F	N	P-F	N-P	N	P	-	G
Fluometuron	F-G	G	F-G	F-G	P-F	N	F-G	N	P	-	N
Pyrithiobac	F	P	-	P-F	F-G	P	G	N	F	9	N
Trifloxsulfuron	-	G	G	E	-	P	G	N	P-F	-	N
Glyphosate	F-G	E	E	E	G	E	G	G	P-G	7	G
Glyphosate + Pyrithiobac	G	E	E	E	G	E	E	G	G	-	G
Glyphosate + Trifloxsulfuron	G	E	-	E	G	E	E	G	P-G	-	G
Glufosinate	F-G	E	-	E	F-G	F-G	G	F-G	P-F	10[5]	F-G

Table VIII-14. Broadleaf Weed Species Control Ratings to Preplant, Preemergence and Postemergence Herbicides in Cotton – Part II (continued)

	Common Broadleaf Weeds[1]										
Product	**PL[2]**	**RW[2]**	**RdW[2]**	**SP[2]**	**SG[2]**	**SN[3]**	**SW[2]**	**TB[3]**	**TSW[2]**	**VL[4]**	**WB[3]**
Postemergence Directed – Layby											
MSMA	P-F	F	N	F	N	N	P	N	F	-	G
Diuron + MSMA	G	E	G-E	G-E	G	F	F	N	G	6[5]	G
Prometryn + MSMA	F-G	E	G	G-E	G	F	F	N	F-G	6[5]	G
Flumioxazin + MSMA	G	G-E	-	G-E	G	F-G	G	N	G-E	9[5]	G
Prometryn/ trifloxysulfuron + MSMA	-	E	-	E	-	F	-	N	F-G	9[5]	N

[1]Weed species: PL = purslane, RW = Common ragweed, RdW = redweed, SP = sicklepod, SG = Spurge, SN = silverleaf nightshade, SW = smartweed, TB = Texas blueweed, TSW = tropical spiderwort, VL = velvetleaf, WB = woolyleaf bursage.
[2](University of Georgia, 2012). Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.
[3]Personal communications with [redacted], Texas A & M University - 2011, Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.
[4] (MSU, 2012). Rating scale: 0-3 = none to slight, 4-6 = fair, 7-8 = good, 9-10 = excellent control.
[5] (University of Arkansas, 2011). Rating Scale: 0 = no control, 10 = 100% control.

VIII.F.2. Herbicide Resistant Weeds in Cotton

Table VIII-15 provides a summary of the common weeds in cotton that have biotypes reported resistant to the various herbicide modes-of-action in the U.S. To date there are only two species with biotypes confirmed to be resistant to dicamba in the U.S. after over 40 years of use – kochia and prickly lettuce (Heap, 2012c). Additionally, a population of lambsquarters has been confirmed as resistant to dicamba in New Zealand, and in Canada, common hempnettle and wild mustard have been confirmed as resistant, for a total of five species worldwide with confirmed resistance to dicamba. Currently in the U.S., six grass species and eight broadleaf species have been confirmed to have resistance to glyphosate. Dicamba provides good to excellent control of all eight of these broadleaf species. None of these broadleaf weed biotypes have been shown to have populations that are resistant to both glyphosate and dicamba. The first species in the U.S. with a biotype resistant to glufosinate was recently confirmed in a glyphosate-resistant Italian Ryegrass population (Avila-Garcia and Mallory-Smith, 2011). Additionally, a population of goosegrass from Malaysia has been confirmed resistant to glufosinate (Seng et al., 2010). Thus, there are a total of two species worldwide with biotypes that have resistance to glufosinate. A discussion regarding the usefulness of MON 88701 in management of herbicide resistant weeds can be found in Section VIII.G., and the potential for development of dicamba and glufosinate resistance in weeds following the introduction of MON 88701 can be found in Appendix I.

Table VIII-15. Common Weeds in Cotton and Weed Resistance to Herbicide Modes of Action in the U.S.[1]

	Mode of Action												
Weed Species	**ACCase Inhibitors**	**ALS Inhibitors**	**Chloroacetamides**	**Dinitroanilines**	**Glycines**	**Organoarsenicals**	**Photosystem II Inhibitors**	**Thiocarbamates**	**Ureas & Amides**	**Synthetic Auxins**	**Bipyridiliums**	**PPO Inhibitors**	**Glutamine Synthase Inhibitors**
Annual Grasses													
Barnyardgrass	X						X	X	X	X			
Crabgrass spp. (large, smooth)	X									X			
Foxtail spp. (giant, green)	X	X		X			X						
Italian ryegrass	X	X	X		X								X
Goosegrass				X	X		X				X		X
Junglerice					X								
Annual Broadleaves													
Black nightshade (Eastern)		X					X						
Common cocklebur		X				X							
Common purslane							X		X				
Common ragweed		X			X		X					X	
Horseweed (marestail)		X			X		X		X		X		
Jimsonweed							X						
Lambsquarters		X					X						
Palmer amaranth		X		X	X		X						
Prickly sida		X											
Pigweed spp. (redroot, smooth, Powell, waterhemp)		X			X		X		X			X	
Russian thistle		X											
Smartweed spp. (Pennsylvania, ladysthumb)							X						
Sunflower		X											
Velvetleaf							X						
Perennial Grasses													
Johnsongrass	X	X		X	X								
Perennial Broadleaves													
Field bindweed										X			

[1](Heap, 2012d)

VIII.G. Introduction of Dicamba and Glufosinate-Tolerant Cotton - MON 88701

VIII.G.1. MON 88701 Product Concept

Monsanto has developed herbicide-tolerant cotton, MON 88701, which will offer growers cotton varieties that are tolerant to both dicamba and glufosinate herbicides. Herbicide tolerances to dicamba and glufosinate were developed due to the benefits associated with these herbicides, including: the ability to control glyphosate-resistant and hard-to-control weeds with two unique modes-of-action and the familiarity growers have with these herbicides. Since dicamba is currently labeled for only preplant applications in cotton, MON 88701 will facilitate a wider window of application for dicamba in cotton by allowing preemergence applications of dicamba up to the day of crop emergence, as well as postemergence in-crop applications up to seven days preharvest. MON 88701 will provide the ability for in-crop postemergence applications of glufosinate from emergence up to the early bloom growth stage, which is the same as the current application timing for glufosinate-tolerant cotton. MON 88701 will be combined with glyphosate-tolerant cotton utilizing traditional breeding techniques. This combination of herbicide-tolerance traits will allow the use of dicamba, glufosinate, and glyphosate herbicides in an integrated weed management program to control a broad spectrum of grass and broadleaf weed species in cotton. These herbicides will provide three distinct modes-of-action for use in conjunction with other herbicide active ingredients and modes-of-action for an effective weed resistance management program in cotton. Dicamba will offer improved in-crop postemergence control of glyphosate's difficult-to-control broadleaf weeds, including Florida pusley, hemp sesbania, lambsquarters, morningglory species, prickly sida, purslane, and Pennsylvania smartweed (Table VIII-16). Glufosinate will offer improved control of certain broadleaf weeds, including lambsquarters, morningglory species, and velvetleaf, as compared to glyphosate. Dicamba and glufosinate will also offer effective control options for glyphosate-resistant broadleaf weed biotypes, including glyphosate-resistant biotypes of Palmer amaranth, marestail, common ragweed, giant ragweed, and waterhemp. Additionally, dicamba and glufosinate will offer an effective control option for broadleaf species resistant to other herbicide classes (*e.g.*, ALS and PPO chemistries).

VIII.G.2. Dicamba and Glufosinate Usage in MON 88701

The current labeled use of dicamba in cotton is limited to early preplant application. Significant restrictions exist in cotton for preplant application of dicamba, including a maximum application rate of 0.25 lbs a.e. per acre, a 21-day interval between application and planting cotton, and a minimum of one inch of rainfall or overhead irrigation before planting cotton to avoid cotton injury (BASF, 2008). To support the introduction of MON 88701, Monsanto will be submitting an application to U.S. EPA to amend Registration Number 524-582, a DGA salt formulation, to allow preemergence and in-crop postemergence dicamba applications to MON 88701. If approved, growers would be authorized to apply dicamba alone or in mixtures with glyphosate, glufosinate, or

other herbicides for preplant or postemergence in-crop applications on MON 88701. Pending EPA registration, dicamba would be authorized to be applied up to 1.0 lb a.e. per acre prior to planting, up to the emergence of cotton, and postemergence in-crop applications, up to 0.5 lbs a.e. per acre each, could be applied up through seven days prior to harvest. These application rates are well within the dicamba rates applied to other crops, such as corn and sugarcane (BASF, 2008). Maximum application amounts for dicamba will be established for total preplant/preemergence applications and in-crop applications with the combined total not to exceed 2.0 lbs a.e. per acre of dicamba per year for all applications. Based on the dicamba label requested by Monsanto, aerial applications of dicamba will not be allowed on MON 88701.

Glufosinate is currently labeled for preplant and in-crop applications in cotton varieties designated as glufosinate-tolerant (Bayer CropScience, 2011). No changes to the glufosinate product labels will be necessary to permit broadcast in-crop applications of glufosinate to MON 88701. Glufosinate can also be applied as a burndown treatment prior to planting or prior to emergence of any conventional or non-glufosinate herbicide-tolerant cotton varieties. Directed postemergence applications are also permitted in non-glufosinate-tolerant varieties, provided no herbicide contacts the cotton foliage. Once MON 88701 is available, growers will be able to apply glufosinate alone or tank-mixed with dicamba for preplant or postemergence in-crop applications on MON 88701. Application rates and timings for glufosinate alone will be the same as currently labeled for glufosinate use in glufosinate-tolerant varieties (*i.e.*, from emergence up to the early bloom stage at 0.402 to 0.530 lbs a.i./acre, seasonal maximum of 1.59 lbs a.i. per acre) (Bayer CropScience, 2011).

The expected use patterns for dicamba and glufosinate on MON 88701 will vary across U.S. cotton growing regions. This variability is dictated by the environment and weed spectrum variations across these regions. The recommendations for the Midsouth and Southeast regions are shown in (Table VIII-16). In these regions, conventional tillage planted acres are expected to receive a single in-crop application per season of dicamba at 0.5 lbs a.e. per acre and conservation tillage or no-tillage acres are expected to receive two applications (one preplant application at 0.375 lbs a.e. per acre and one in-crop application at 0.50 lbs a.e. per acre). All acres in this region where glyphosate-resistant weeds are present, regardless of tillage, are expected to receive a single in-crop application of glufosinate as 0.53 lbs a.i. per acre. For the remaining acres where glyphosate-resistant weeds are not present, glyphosate will likely be used for control of late-emerging weeds. Dicamba and glufosinate use in eastern Texas, is expected to be similar to that described for the Midsouth and Southeast regions.

Table VIII-16. Anticipated Weed Management Recommendations for MON 88701 Combined with Glyphosate-Tolerant Cotton Systems for MO, AR, TN, AL, FL, GA, NC, SC, VA, LA, MS and eastern TX[1]

Application Timing	Conventional Tillage		Conservation Tillage (No-till or reduced till)	
	No GR Weeds[2]	GR Weeds or Suspected GR Weeds[2]	No GR Weeds[2]	GR Weeds or Suspected GR Weeds[2]
Preemergence (burndown, at planting)[3]	Residual	Residual	Dicamba + Glyphosate + Residual	Dicamba + Glyphosate + Residual
Postemergence[3]	Dicamba + Glyphosate	Dicamba + Glyphosate + Residual	Dicamba + Glyphosate + Residual	Dicamba + Glyphosate + Residual
Postemergence[3]	Glyphosate	Glufosinate	Glyphosate	Glufosinate + Residual

[1] The anticipated use patterns represent a high-end estimate for predicting dicamba use associated with MON 88701 combined with glyphosate-tolerant cotton. Actual weed control practices by growers will vary depending on the specific weed spectrum and agronomic situation of the individual cotton field, specifically dicamba use could be lower especially for the preemergence and second postemergence applications.

[2] Recommendations for all fields in these regions will assume GR weeds are present.

[3] Monsanto and academics recommend the use of soil residuals as part of a comprehensive weed resistance management program to ensure that two effective herbicide modes-of-action are used in cotton and to provide protections against additional resistance development to existing cotton herbicides.

In western Texas, New Mexico, Kansas, Oklahoma, California and Arizona, dicamba is expected to be utilized more extensively than glufosinate for management of hard-to-control and/or glyphosate-resistant weeds in MON 88701. Glufosinate is considered less effective on the weed spectrum under the high temperature and low humidity environmental conditions in these regions (Bayer CropScience, 2011). The recommendations for these cotton growing areas are shown in (Table VIII-17**)**. All acres are expected to receive one preplant application of dicamba (0.375 lbs a.e. per acre). Areas with glyphosate-resistant weeds are also expected to receive two in-crop applications of dicamba (0. 50 lbs a.e./acre) per season, whereas areas without glyphosate-resistant weeds will only receive one in-crop application of dicamba (0.50 lbs a.e./acre).

Table VIII-17. Anticipated Weed Management Recommendations for MON 88701 Combined with Glyphosate-Tolerant Cotton Systems for western TX, NM, KS, OK, CA, and AZ [1]

Application Timing	Conventional Tillage		Conservation Tillage (No-till or reduced till)	
	No GR Weeds	GR Weeds or Suspected GR Weeds	No GR Weeds	GR Weeds or Suspected GR Weeds
Preemergence (burndown, at planting) [2]	Dicamba + Glyphosate + Residual	Dicamba + Glyphosate + Residual	Dicamba + Glyphosate + Residual	Dicamba + Glyphosate + Residual
Postemergence[2]	Dicamba + Glyphosate	Dicamba + Glyphosate	Dicamba + Glyphosate	Dicamba + Glyphosate
Postemergence[2]	Glyphosate	Dicamba + Glyphosate	Glyphosate	Dicamba + Glyphosate

[1] The anticipated use patterns represent a high-end estimate for predicting dicamba use associated with MON 88701 combined with glyphosate-tolerant cotton. Actual weed control practices by growers will vary depending on the specific weed spectrum and agronomic situation of the individual cotton field, specifically dicamba use could be lower especially for the preemergence and second postemergence applications.

[2] Monsanto and academics recommend the use of soil residuals as part of a comprehensive weed resistance management program to ensure that two effective herbicide modes-of-action are used in cotton and to provide protections against additional resistance development to existing cotton herbicides.

VIII.G.3. MON 88701 in Combination with Glyphosate-Tolerant Cotton Systems

With the introduction of MON 88701 into glyphosate-tolerant systems, growers will continue to be able to use dicamba, glufosinate, and glyphosate herbicides for preplant burndown, without the plant-back restrictions currently in place. Tables VIII-10 and VIII-12 show weed control ratings for glyphosate, glufosinate, glyphosate tank-mixed with dicamba, and glufosinate tank-mixed with dicamba compared to other herbicide regimes when applied as a preplant burndown application to common broadleaf weed species found in fields prior to planting cotton. Glyphosate alone provides excellent control of many grass species and is superior to glufosinate on many grass species. Therefore, glufosinate applications in MON 88701 are not expected to provide improvement in control of grass species in no-till systems, except where glyphosate-resistant grass species may be present. Certain hard-to-control broadleaf weeds such as curly dock, Carolina geranium, cutleaf primrose, and wild radish, are difficult to control with glyphosate. Similarly, glufosinate provides unsatisfactory control of certain

broadleaf weeds such as Carolina geranium, cutleaf primrose, and henbit. Tank-mixing dicamba with glyphosate or glufosinate improves the control of the difficult-to-control weed species for both of these products. The dicamba tank mix combinations will also provide excellent control of glyphosate-resistant marestail and fair to good control of glyphosate-resistant Palmer amaranth. Dicamba will be complementary to glyphosate or glufosinate for preplant burndown weed control in cotton and will offer growers equal or superior weed control to other preplant herbicides or herbicide tank mixtures. MON 88701 will provide additional application flexibility with dicamba allowing applications up to the day of planting in cotton. In addition to complementing the weed control of glyphosate, dicamba and glufosinate will provide additional modes-of-action in the glyphosate-tolerant cotton systems to lower the potential risk of weed species developing resistance to glyphosate. Furthermore, dicamba and glufosinate will provide alternative modes-of-action for control of broadleaf weeds with populations known to be resistant to glycine, ALS, and PPO classes of herbicides (see Table VIII-15).

Upon integration of MON 88701 into glyphosate-tolerant cotton systems, in-crop postemergence applications of dicamba, glufosinate, and glyphosate herbicides will be permitted in cotton production. Tables VIII-18 and VIII-19 illustrate common broadleaf weed responses to dicamba, glyphosate, glufosinate, and several other labeled in-crop over-the-top herbicide treatments in cotton. Since dicamba is not currently labeled for in-crop applications in cotton, weed control ratings for dicamba were taken from labeled in-crop applications of dicamba in corn for comparison purposes. Glyphosate provides good to excellent control of all the listed annual grasses and most of the annual broadleaf weeds. When compared to glufosinate, glyphosate provides better control of some of the annual grasses (broadleaf signalgrass, crabgrass, crowsfootgrass, foxtail, goosegrass, seedling johnsongrass, Texas panicum), perennial grasses (johnsongrass and bermudagrass), and some of the broadleaf weeds (devil's claw, Florida beggarweed, Florida pusley, pigweed species, and silverleaf nightshade). However, glufosinate data illustrates slightly better control of some of the broadleaf weeds compared to glyphosate, namely lambsquarter, morningglory, and velvetleaf. Dicamba as a tank mixture will complement the weed control of in-crop application(s) of either glyphosate or glufosinate. The use of dicamba will improve control of most of the broadleaf weeds where either glyphosate or glufosinate provide unsatisfactory control, with the exception of spurge, silverleaf nightshade, Texas blueweed, tropical spiderwort, velvetleaf, and woolyleaf bursage. Other herbicide treatments are available when needed to provide effective control of these weed species. In addition, an in-crop application of dicamba in combination with either glyphosate or glufosinate will assist in the management of glyphosate-resistant weed biotypes.

Currently, many residual and non-residual herbicides are used in combination with glyphosate in preplant and in-crop postemergence applications in cotton. The addition of dicamba and glufosinate to the system are expected to offer increased benefits over the current alternative herbicides as supplements to glyphosate for preplant and in-crop applications on MON 88701, including increased flexibility and reduced crop injury.

Since planting interval restrictions following preplant applications of dicamba in cotton will be removed, dicamba will have greater flexibility for preplant applications than current preplant applications of 2,4-D and will potentially replace some 2,4-D applications in cotton. The broadleaf weed control provided by dicamba and glufosinate, plus the crop tolerance when applied to MON 88701, will allow the potential replacement of some other in-crop alternative herbicides used for broadleaf weed control in cotton, particularly diuron, fomesafen, fluometuron, and paraquat. Considering the characteristics of dicamba and glufosinate from the perspective of weed control and compatibility with glyphosate, it is concluded that MON 88701 will complement the established safety and efficacy of glyphosate use in glyphosate-tolerant cotton systems.

VIII.G.4. MON 88701 as a Weed Resistance Management Tool

Although herbicide resistance may eventually occur in a weed species when an herbicide is widely used, resistance can be delayed, contained, and managed through research, education, and good management practices. The addition of dicamba and glufosinate tolerance to the glyphosate-tolerant cotton systems will provide an efficient method for incorporation of additional modes-of-action in the system, and reduce the potential for further resistance development to glyphosate, dicamba, and glufosinate, as well as other important cotton herbicides. Current research, conducted by Monsanto, to define the optimum weed management systems indicate the following: 1) in MO, AR, TN, AL, FL, GA, NC, SC, VA, LA, MS, and eastern TX, the recommendation will be to apply a soil-active residual herbicide followed by an in-crop early postemergence application of dicamba tank-mixed with glyphosate, and a residual product, followed by a late post-emergence application of glufosinate tank-mixed with a residual product (Table VIII-16**)**; and 2) in western TX, KS, OK, NM, AZ, and CA, the recommendation will be to apply a soil-active residual herbicide, followed by an in-crop postemergence application of dicamba tank-mixed with glyphosate at early and late postemergence (Table VIII-17**)**. This will ensure the use of more than one mode-of-action against the targeted species. In both areas, a preplant application of dicamba tank-mixed with glyphosate may be recommended, in addition to the in-crop applications described above. This is not expected to increase selection pressure on either product since the preplant weed spectrum is generally different from the in-crop spectrum.

Stewardship of dicamba and glufosinate to preserve their usefulness for growers is an important aspect of Monsanto's stewardship commitment, as is discussed in Appendix I. Specifically, Monsanto has implemented and will continue to develop and proactively provide weed resistance management practices[6], and will utilize multiple methods to distribute technical and stewardship information to growers, academics, and grower advisors through a variety of communication tools. Monsanto's Technology Use Guide

[6] Weed resistance management guidelines available at http://www.weedtool.com and http://www.monsanto.com/weedmanagement/Pages/default.aspx

(TUG) will set forth the requirements and best practices for the cultivation of MON 88701 including recommendations on weed resistance management practices. Growers purchasing products containing MON 88701 are required by the Monsanto Technology Stewardship Agreement (MTSA) to read and follow the TUG. Furthermore, Monsanto is committed to actively evaluate herbicide performance and weed efficacy on a continuing basis, and develop additional mitigation plans as necessary to manage resistance development for glyphosate, dicamba, and glufosinate.

VIII.G.5. Introduction of Dicamba and Glufosinate-Tolerant Cotton - MON 88701 - Conclusion

Integration of MON 88701 into glyphosate-tolerant cotton systems will allow the use of dicamba, glufosinate, and glyphosate herbicides in an integrated weed management program to control a broad spectrum of grass and broadleaf weed species in cotton. These herbicides will also provide three distinct modes-of-action for an effective proactive and reactive weed resistance management program in cotton. Due to the crop safety of MON 88701 to dicamba and glufosinate, growers will be afforded two effective herbicide modes-of-action for in-crop control of glyphosate's hard-to-control and resistant broadleaf weeds that are present in U.S. cotton production.

Furthermore, the integration of MON 88701, along with the glyphosate-tolerant cotton systems, will provide growers with the ability to continue use of established cotton production practices including tillage systems; the same planting and harvesting machinery; traditional management of insects, diseases, and other pests; and many of the current herbicides used for weed control, including glyphosate with its established environmental and grower benefits. Therefore, it is anticipated that the commercialization of MON 88701 in the U.S. is not likely to impact current cotton agronomic practices, cultivation or seed production practices, beyond the intended benefits of more effective and improved management of common and troublesome weeds, including herbicide-resistant weeds.

Table VIII-18. Responses of Common Broadleaf Weeds to Dicamba and Glufosinate Compared to Labeled Postemergence Herbicides in Cotton Production – Part I

	Common Broadleaf Weeds[1]												
Product	**CB[2]**	**DC[3]**	**FB[2]**	**FP[2]**	**GC[4]**	**HS[2]**	**NS[3]**	**JW[2]**	**LQ[2]**	**MG[2]**	**PA[2]**	**PW[2]**	**PS[2]**
Postemergence Over-The-Top													
Dicamba	E[5]	G-E	G[5]	G[5]	C[4]	E[5]	C[4]	E[5]	E[5]	E[5]	G-E[5]	G-E[5]	E[5]
MSMA	E	P-F	E	N-P	P	-	P	P	P	P-F	P	P	P
Fluometuron	F-G	F-G	G	P-F	-	-	-	G	G	G	P-F	F	F-G
Pyrithiobac	G	G	G	N-P	P	G-E	C	E	N	G	F	G	F
Trifloxsulfuron	G-E	G	G-E	P	-	-	-	N	G	G	P-F	F-G	N
Glyphosate	E	E	E	P-G	C	P-F	C	E	G	F-G	E	E	F-G
Glyphosate + Pyrithiobac	E	E	E	P-G	-	G-E	-	E	G	G-E	E	E	G
Glyphosate + Trifloxsulfuron	E	E	E	P-G	-	-	-	E	E	E	E	E	G
Glufosinate	E	G-E	G	F	C	-	C	E	E	E	F-G	G	F

[1] Weed species: CB = common cocklebur, DC = devil's claw, FB = Florida beggarweed, FP = Florida pusley, GC = ground cherry, HS = hemp sesbania, NS = nightshade, JW = jimsonweed, LG = Common lambsquarters, MG = morningglory species, PA = Palmer amaranth, PW = pigweed species, PS = Prickly sida.

[2] (University of Georgia, 2012). Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.

[3] Personal communications with [redacted] Texas A & M University - 2011, Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.

[4] (University of California, 2012). Ratings Key: C = control, P = partial control, N = no control, - = no information.

[5] (University of Georgia, 2010). Weed control ratings key: E = Excellent control, 90% or above; G = Good control, 80% or above; F = Fair control, less than 80% control; P = Poor control.

Table VIII-19. Responses of Common Broadleaf Weeds to Dicamba and Glufosinate Compared to Labeled Postemergence Herbicides in Cotton Production – Part II

	Common Broadleaf Weeds[1]										
Product	**PL[2]**	**RW[2]**	**RdW[2]**	**SP[2]**	**SG[2]**	**SN[3]**	**SW[2]**	**TB[3]**	**TSW[2]**	**VL[4]**	**WB[3]**
Postemergence Over-The-Top											
Dicamba	E[5]	E[5]	-	E[5]	P[6]	F[3]	E[5]	F[3]	P[5]	F-G[5]	F[3]
MSMA	P-F	P-F	N	P-F	N	P-F	N-P	N	P	-	G
Fluometuron	F-G	G	F-G	F-G	P-F	N	F-G	N	P	-	N
Pyrithiobac	F	P	G	P-F	F-G	P	G	N	F	9	N
Trifloxsulfuron	-	G	G	E	-	P	G	N	P-F	-	N
Glyphosate	F-G	E	E	E	G	E	G	G	P-G	7	G
Glyphosate + Pyrithiobac	G	E	E	E	G	E	E	G	G	-	G
Glyphosate + Trifloxsulfuron	G	E	-	E	G	E	E	G	P-G	-	G
Glufosinate	F-G	E	-	E	F-G	F-G	G	F-G	P-F	10[7]	F-G

[1] Weed species: PL = purslane, RW = Common ragweed, RdW = redweed, SP = sicklepod, SG = Spurge, SN = silverleaf nightshade, SW = smartweed, TB = Texas blueweed, TSW = tropical spiderwort, VL = velvetleaf, WB = woolyleaf bursage.

[2] (University of Georgia, 2012). Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.

[3] Personal communications with [redacted], Texas A & M University, Weed control ratings key: E = 90% or better control; G = 80% to 90% control; F = 60%-80% control; P = 30% to 60% control; N = < 30% control.

[4] (MSU, 2012). Rating scale: 0-3 = none to slight, 4-6 = fair, 7-8 = good, 9-10 = excellent control.

[5] (University of Georgia, 2010). Weed control ratings key: E = Excellent control, 90% or above; G = Good control, 80% or above; F = Fair control, less than 80% control; P = Poor control.

[6] (University of California, 2012). Ratings Key: C = control, P = partial control, N = no control, - = no information.

[7] (University of Arkansas, 2011). Rating Scale: 0 = no control, 10 = 100% control.

VIII.H. Crop Rotation Practices in Cotton

The rotation of cotton with other crops is an integral part of most farm management programs across the southern United States cotton growing region. Ideally, cotton should be rotated with other crops on a regular basis to maintain soil productivity and reduce the incidence of various weeds, insect pests, or diseases (Hake et al., 1996d). Rotating cotton with grass crops such as corn helps to reduce the soil inoculum level of the seedling disease fungi *Pythium* and *Rhizoctonia*. These seedling diseases can increase in continuous cotton cropping systems. Crop rotations or the lack of rotations, along with weed control programs used in these crops, can play an important factor on the weed spectrum (Smith and Cothren, 1999). In addition, the crop rotation and weed control programs can increase or decrease the populations of certain weed species. Production costs, relative rate of return, and the current market conditions will dictate which crops to rotate with cotton or whether to grow continuous cotton. These economic factors may outweigh the agronomic benefits of crop rotation. According to Sandretto and Payne (2006) statistics, cotton was grown in a continuous cropping system on 73% of the acreage in the major cotton growing states in 2003. Cotton was rotated with other row crops such as corn or soybean on about 20% of the acreage.

Crop rotations for cotton vary from region to region and state to state and often within a state. This section provides a detailed description and quantitative assessment by state of the rotational cropping practices immediately following cotton production. This assessment accounts for about 99% of the total cotton acreage. These data are presented in Tables VIII-20 through VIII-24). Seventeen crops immediately follow cotton in the crop rotation sequence according to this assessment. In the U.S., approximately 54% of the cotton acres are followed by cotton in the crop rotation sequence. Corn (16%), soybean (8%), sorghum (8%), wheat (9%), and peanuts (4%) are the other crops most frequently following cotton. The other crops following cotton are 0.5% or less of the cotton acres.

Grower survey data available to Monsanto (2011) for dicamba, glufosinate, and glyphosate herbicide usage were utilized for this assessment. For the purpose of this assessment, a 50% adoption rate in U.S. cotton and soybean production was assumed for both MON 88701 and MON 87708 (dicamba-tolerant soybean), respectively. In the following data tables, columns F, H, and J provide the number of acres of dicamba, glufosinate, glyphosate that follow cotton in the rotation crops, respectively. Columns K, L, and M provide the percentage of dicamba, glufosinate, and glyphosate usage in the total rotational crop acreage (*i.e.*, the percentage of cotton acres where dicamba, glufosinate and glyphosate, respectively, are used in the subsequent crop). For the entire U.S. (Table VIII-20), 33.1%, 11.9%, and 75.0% of the rotational crop acreage would be treated with dicamba, glufosinate, and glyphosate, respectively. The percentage of dicamba usage in the rotation would be the highest in the Southeast region (35.8%) and lowest in the West region (17.9%). MO (45.6%) and OK (48.0%) would be the states with the highest dicamba usage in the rotation and NM (5.8%) and AZ (3.2%) the lowest. The percentage of glufosinate usage in the rotation would be the highest in the Southeast (25.7%) and Midsouth (21.4%) regions and lowest in the Southwest region (2.5%). MO (43.0%) and OK (45.1%) would be the states with the highest glufosinate usage in the

rotation and LA (1.8%), AZ (0%), and NM (0.2%) the lowest. The percentage of glyphosate usage in the rotation would be the highest in the Midsouth region (89.9%) and lowest in the West region (37.8.0%). MO (95.3%) would have the highest glyphosate usage in the rotation and AZ (19.7%) the lowest. In the Southwest region where almost 55% of the cotton is grown, 33.1%, 2.5%, and 70.4% of the rotational crop acres following cotton would be treated with dicamba, glufosinate, and glyphosate, respectively.

Table VIII-20. Rotational Practices in the U.S. Following Cotton Production

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres[2]	% Rotational Crop of Total Cotton[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
United States 10,974	Cotton	5858	53.4	50	2930	21.	1264	90.2	5284	26.7	11.5	48.2
	Corn	1736	15.8	8.1	141	6	37	88.0	1527	1.3	0.3	13.9
	Soybean	861	7.8	50	431	2.1	8	95.9	826	3.9	0.1	7.5
	Sorghum	836	7.6	8.3	69	1.0		34.7	290	0.6		2.6
	Wheat	1025	9.3	5.6	57	NL		14.1	145	0.5		1.3
	Barley	40	0.4	5.0		NL		27.5	11	0.02		0.1
	Peanut	432	3.9	NL		NL		21.1	91			0.8
	Sunflower	22	0.2	NL		NL		72.7	16			0.1
	Alfalfa[8]	47	0.4	NL		NL		50	24			0.2
	Vegetables[9]	50	0.5	NL		NL		2	1			0.01
	Dry Beans	0.5	0.005	NL		NL		40.0	0.2			0.002
	Peppers	8	0.1	NL		NL		37.5	3			0.03
	Tomatoes	24	0.2	NL		NL		45.8	11			0.1
	Onions	6	0.06	NL		NL		33.3	2			0.02
	Tobacco	30	0.3	NL		NL		NL				
		Total:[10] 10,974			**Total: 3,630**	NL	**Total: 1,309**		**Total: 8,231**	**33.1**	**11.9**	**75.0**

This table was developed by compiling the data from all four regional summaries (Tables VIII-21 through VIII-24). All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

[1]Cotton acreage based on 2010 planting data (USDA-NASS, 2011e).

[2]Column C is obtained by compiling the data from the four regional summaries.

[3]Column D is obtained by dividing Column C by Column A.

[4]Column E is obtained by dividing Column F by Column C; Column F is obtained by compiling the data from all four regional summaries.

[5]Column G is obtained by dividing Column H by Column C; Column H is obtained by compiling the data from all four regional summaries.

[6]Column I is obtained by dividing Column J by Column C; Column J is obtained by compiling the data from all four regional summaries

[7] Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total; Column M is obtained by dividing Column J Total by Column C Total.

[8]Newly seeded alfalfa.

[9]Vegetables: Cauliflower (37k acres), lettuce (271 k acres), and broccoli (124k acres) (USDA-NASS, 2011d).

[10] Totals may not be exact due to rounding.

Table VIII-21. Rotational Practices Following Cotton Production in the Southeast Region

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres Following Cotton[2]	% Rotational Crop Acres[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region 2597	Cotton	1311	50.5	50	656	50	656	95.1	1247	25.2	25.2	48.0
	Corn	340	13.1	6.2	21	2.3	8	88.3	300	0.8	0.3	11.5
	Soybean	507	19.5	50	254	0.9	4	97.8	496	9.8	0.2	19.1
	Peanut	410	15.8	NL		NL		20.9	86			3.3
	Tobacco	30	1.1	NL		NL		NL				
		Total:[10] 2597			**Total: 930**		**Total: 668**		**Total: 2128**	**35.8**	**25.7**	**81.9**
AL 340	Cotton	102	30	50	51	50	51	97	99	15.0	15.0	29.1
	Corn	68	20	7[8]	5	2[8]	1	97	66	1.4	0.4	19.4
	Soybean	119	35	50	60	1[8]	1	100	119	17.5	0.4	35.0
	Peanut	51	15	NL		NL		30	15			4.5
		Total: 340			**Total: 115**		**Total: 54**		**Total: 299**	**33.9**	**15.8**	**88.0**
FL 92	Cotton	46	50	50	23	50	23	99	46	25.0	25.0	49.5
	Corn	9	10	7[8]	1	2[8]	1	78[8]	7	0.7	1.1	7.8
	Soybean	5	5	50	2	1[8]	0.05	94[8]	4	2.5	0.1	4.7
	Peanut	32	35	NL		NL		27	9			9.5
		Total: 92			**Total: 26**		**Total: 24**		**Total: 66**	**28.2**	**26.1**	**71.5**
GA 1330	Cotton	798	60	50	399	50	399	93	742	30.0	30.0	55.8
	Corn	133	10	7[8]	9	2[8]	3	92	122	0.7	0.2	9.2
	Soybean	133	10	50	67	1[8]	1	99	132	5.0	0.1	9.9
	Peanut	266	20	NL		NL		18	48			3.6
		Total: 1330			**Total: 475**		**Total: 403**		**Total: 1044**	**35.7**	**30.3**	**78.5**

Table VIII-21. Rotational Practices Following Cotton Production in the Southeast Region (continued)

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres[2]	% Rotational Crop of Total Cotton[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
NC	Cotton	231	42	50	116	50	116	99	229	21.0	21.0	41.6
550	Corn	83	15	7[8]	3	2[8]	2	79	65	0.5	0.4	11.9
	Soybean	165	30	50	83	1[8]	1	95	157	15.0	0.2	28.5
	Peanut	44	8	NL		NL		23[8]	10			1.8
	Tobacco	28	5	NL		NL		NL				
		Total: 550			**Total: 201**		**Total: 119**		**Total: 461**	**36.5**	**21.5**	**83.8**
SC	Cotton	101	50	50	51	50	51	100	101	25.0	25.0	50.0
202	Corn	30	15	7[8]	2	2[8]	1	86	26	1.1	0.3	12.9
	Soybean	61	30	50	30	1[8]	1	98	59	15.0	0.3	29.4
	Peanut	8	4	NL		NL		23[8]	2			0.9
	Tobacco	2	1	NL		NL						
		Total: 202			**Total: 83**		**Total: 52**		**Total: 188**	**41.1**	**25.6**	**93.2**
VA	Cotton	33	40	50	17	50	17	91	30	20.0	20.0	36.4
83	Corn	17	20	7[8]	1	2[8]	0.3	79	13	1.4	0.4	15.8
	Soybean	25	30	50	12	1[8]	0.2	99	25	15.0	0.3	29.7
	Peanut	8	10	NL		NL		23[8]	2			2.3
		Total: 83			**Total: 30**		**Total: 17**		**Total: 70**	**36.4**	**20.7**	**84.2**

The Southeast region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

[1]Cotton acreage based on 2010 planting data (USDA-NASS, 2011e).

[2]Column C is obtained by multiplying Column A by Column D.

[3]The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist and Extension Weed Control Specialist in cotton ([redacted] Auburn University; [redacted] University of Florida; [redacted] University of Georgia; [redacted] Clemson University; and [redacted] Virginia Tech University, Personal Communications, November, 2010).

[4]Dicamba usage data in Column E except for cotton and soybean is based on 2010 data (Monsanto Company, 2011). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

[5]Glufosinate usage data in Column G except for cotton is based on 2010 (Monsanto Company, 2011). Glufosinate usage in cotton (50%) is the future market adoption estimate.

[6]Glyphosate usage data in Column I is based on 2010 data (Monsanto Company, 2011).

[7]Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

[8]Since no data was reported or the survey sample size was too small for the herbicide data in the state to be statistically reliable, the percent usage for the herbicide/crop in the U.S. was used.

[10]Totals may not be exact due to rounding.

Table VIII-22. Rotational Practices Following Cotton Production in the Midsouth Region

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres[2]	% Rotational Crop of Total Cotton[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region 1920	Cotton	786	40.9	50	393	50	393	99	780	20.5	20.5	40.6
	Wheat	59	3.0	6.0	4	NL		15	9	0.2		0.5
	Corn	711	37.0	10.1	72	2.1	15	85	607	3.7	0.8	31.6
	Soybean	337	17.6	50	169	1.0	4	94	317	8.8	0.2	16.5
	Sorghum	27	1.4	12	3	NL		45	12	0.2		0.6
		Total: [9] 1920			**Total: 640**		**Total: 411**		**Total: 1725**	**33.3**	**21.4**	**89.9**
AR 545	Cotton	218	40	50	109	50	109	100	218	20.0	20.0	40.0
	Corn	164	30	7[8]	16	2[8]	3	78[8]	128	2.9	0.6	23.4
	Soybean	136	25	50	68	1[8]	1	87	119	12.5	0.3	21.8
	Sorghum	27	5	12[8]	3	NL		45[8]	12	0.6		2.3
		Total: 545			**Total: 196**		**Total: 114**		**Total: 476**	**36.0**	**20.9**	**87.4**
LA 255	Cotton	0	0	50	0	50	0	100	0			
	Corn	191	75	7[8]	13	2[8]	4	78	149	5.3	1.5	58.5
	Soybean	64	25	50	32	1[8]	1	100	64	12.5	0.3	25.0
		Total: 255			**Total: 45**		**Total: 4**		**Total: 213**	**17.8**	**1.8**	**83.5**
MS 420	Cotton	168	40	50	84	50	84	99	166	20.0	20.0	39.6
	Corn	189	45	7[8]	13	2[8]	4	98	185	3.2	0.9	44.1
	Soybean	63	15	50	32	1[8]	1	99	62	7.5	0.2	14.9
		Total: 420			**Total: 129**		**Total: 88**		**Total: 414**	**30.7**	**21.1**	**98.6**

Table VIII-22. Rotational Practices Following Cotton Production in the Midsouth Region (continued)

A	B	C	D	E	F	G	H	I	J	K	L	
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres[2]	% Rotational Crop of Total Cotton[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
MO	Cotton	264	85	50	132	50	132	100	264	42.5	42.5	85.0
310	Corn	31	10	7[8]	2	4	1	59	18	0.6	0.4	5.9
	Soybean	16	5	50	8	2	0.3	88	14	2.5	0.1	4.4
		Total: 310			**Total: 142**		**Total: 133**		**Total: 295**	**45.6**	**43.0**	**95.3**
TN	Cotton	137	35	50	68	50	68	97	132	17.5	17.5	34.0
390	Wheat	59	15	6[8]	4	NL		15[8]	9	0.9		2.3
	Corn	137	35	11	27	2[8]	3	93	127	6.9	0.7	32.6
	Soybean	59	15	50	29	1[8]	1	100	59	7.5	0.2	15.0
		Total: 390			**Total: 128**		**Total: 72**		**Total: 327**	**32.8**	**18.4**	**83.8**

The Midsouth region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

[1] Cotton acreage based on 2010 planting data (USDA-NASS, 2011e).

[2] Column C is obtained by multiplying Column A by Column D.

[3] The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist and Extension Weed Control Specialist in cotton ([redacted] University of Arkansas; [redacted] Louisiana State University; [redacted] Mississippi State University; [redacted] University of Missouri; and [redacted] University of Tennessee, Personal Communications November, 2010).

[4] Dicamba usage data in Column E except for cotton and soybean is based on 2010 data (Monsanto Company, 2011). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

[5] Glufosinate usage data in Column G except for cotton is based on 2010 (Monsanto Company, 2011). Glufosinate usage in cotton (50%) is the future market adoption estimate.

[6] Glyphosate usage data in Column I is based on 2010 data (Monsanto Company, 2011).

[7] Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

[8] Since no data was reported or the survey sample size was too small for the herbicide data in the state to be statistically reliable, the percent usage for the herbicide/crop in the U.S. was used.

[9] Totals may not be exact due to rounding.

Table VIII-23. Rotational Practices Following Cotton Production in the Southwest Region

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres[2]	% Rotational Crop Total Cotton[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region 5953	Cotton	3607	60.6	50	1804	3.8	138	86.9	3136	30.3	2.3	52.7
	Wheat	765	12.9	5.3	41	NL		13.9	106	0.7		1.8
	Corn	685	11.5	7.0	48	2.0	14	90.5	620	0.8	0.2	10.4
	Soybean	17	0.3	50	8	1.0	0.2	78.2	13	0.1	0.003	0.2
	Sorghum	808	13.6	8.2	66	NL		34.4	278	1.1		4.7
	Dry Bean	0.5	0.01	NL		NL		35.0	0.2			0.003
	Alfalfa[8]	18	0.3	NL		NL		50.0	9			0.1
	Peanuts	22	0.4	NL		NL		23.0	5			0.1
	Sunflower	22	0.4	NL		NL		74.0	16			0.3
	Peppers	8	0.1	NL		NL		43.0	3			0.1
		Total: [10] 5953			**Total: 1967**		**Total: 152**		**Total: 4188**	**33.1**	**2.5**	**70.4**
KS 51	Cotton	3	5.0	50	1	50	1	100	3	2.5	2.5	5.0
	Wheat	13	25.0	13	2	NL		8	1	3.3		2.0
	Corn	20	40.0	11	2	2[9]	0.4	88	18	4.2	0.8	35.2
	Soybean	3	5.0	50	1	1[9]	0.03	96	2	2.5	0.1	4.8
	Sorghum	13	25.0	19	2	NL		57	7	4.7		14.3
		Total: 51			**Total: 9**		**Total: 2**		**Total: 31**	**17.2**	**3.4**	**61.3**

Table VIII-23. Rotational Practices Following Cotton Production in the Southwest Region (continued)

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres[2]	% Rotational Crop of Total Cotton[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
NM	Cotton	3	5.0	50	1	0	0	91[9]	2	2.5		4.6
50	Wheat	12	24.0	6[9]	0.7	NL		15[9]	2	1.4		3.6
	Corn	5	10.0	7[9]	0.4	2[9]	0.1	78[9]	4	0.7	0.2	7.8
	Sorghum	5	10.0	12[9]	1	NL		45[9]	2	1.2		4.5
	Dry Beans	0.5	1.0	NL		NL		35[9]	0.2			0.4
	Alfalfa[8]	18	35.0	NL		NL		50	9			17.5
	Peppers	8	15.0	NL		NL		43[9]	3			6.5
		Total: 50			**Total: 3**		**Total: 0.1**		**Total: 22**	**5.8**	**0.2**	**44.8**
OK	Cotton	257	90	50	128	50	128	99	254	45.0	45.0	89.1
285	Corn	9	3	7[9]	0.6	2[9]	0.2	69	6	0.2	0.1	2.1
	Soybean	14	5	50	7	1[9]	0.1	75	11	2.5	0.1	3.8
	Sorghum	6	2	12[9]	0.7	NL		35	2	0.2		0.7
		Total: 285			**Total: 137**		**Total: 129**		**Total: 273**	**48.0**	**45.1**	**95.6**
TX	Cotton	3346	60.1	50	1673	0.25	8	86	2877	30.1	0.2	51.7
5567	Wheat	740	13.3	5	39	NL		14	104	0.7		1.9
	Corn	651	11.7	7	45	2[9]	13	91	593	0.8	0.2	10.6
	Sorghum	785	14.1	8	63	NL		34	267	1.1		4.8
	Peanuts	22	0.4	NL		NL		23[9]	5			0.1
	Sunflower	22	0.4	NL		NL		74[9]	16			0.3
		Total: 5567			**Total: 1819**		**Total: 21**		**Total: 3862**	**32.7**	**0.4**	**69.4**

The Southwest region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

[1]Cotton acreage based on 2010 planting data (USDA-NASS, 2011e).

[2]Column C is obtained by multiplying Column A by Column D.

[3]The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist and Extension Weed Control Specialist in cotton ([redacted] Kansas State University; [redacted] New Mexico State University; [redacted] Oklahoma State University; [redacted] Texas A & M University; [redacted] Texas A & M University, Personal Communications November, 2010).).

[4]Dicamba usage data in Column E except for cotton and soybean is based on 2010 data (Monsanto Company, 2011). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

[5]Glufosinate usage data in Column G except for cotton is based on 2010 (Monsanto Company, 2011). Glufosinate usage in cotton (50%) is the future market adoption estimate for most of the Southwest region. No glufosinate usage is expected in NM and West TX.

[6]Glyphosate usage data in Column I is based on 2010 data (Monsanto Company, 2011).

[7]Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

[8] Newly seeded alfalfa; Glyphosate usage in alfalfa (50%) is future market adoption estimate.

[9]Since no data was reported or the survey sample size was too small for the herbicide data in the state to be statistically reliable, the percent usage for the herbicide/crop in the U.S. was used.

[10]Totals may not be exact due to rounding.

Table VIII-24. Rotational Practices Following Cotton Production in the West Region

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Cotton Acres[1]	Rotational Crops Following Cotton	Rotational Crop Acres[2]	% Rotational Crop of Total Cotton[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop Option[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region 504	Cotton	153	30.4	50.0	77	50.0	77	79.0	121	15.2	15.2	24.0
	Wheat	202	40.0	6.0	12	NL		15.0	30	2.4		6.0
	Barley	40	7.9	4.0	2	NL		27.0	11	0.3		2.1
	Alfalfa[8]	30	5.9	NL		NL		50.0	15			2.9
	Tomatoes	24	4.9	NL		NL		43.0	11			2.1
	Onions	6	1.2	NL		NL		27.0	2			0.3
	Vegetables[9]	50	9.8	NL		NL		3.0	1			0.3
		Total: [11] 504			**Total: 90**		**Total: 77**		**Total: 190**	**17.9**	**15.2**	**37.8**
AZ 198	Wheat	79	40	6[10]	5	NL		15[10]	12	1.6		6.0
	Barley	40	20	4[10]	2	NL		27[10]	11	0.5		5.4
	Alfalfa[8]	30	15	NL		NL		50	15			7.5
	Vegetables[9]	50	25	NL		NL		3[10]	1			0.8
		Total: 198			**Total: 6**		**Total: 0**		**Total: 39**	**3.2**	**0.0**	**19.7**
CA 306	Cotton	153	50	50	77	50	77	79	121	25.0	25.0	39.5
	Wheat	122	40	6[10]	7	NL		15[10]	18	2.4		6.0
	Tomatoes	24	8	NL		NL		43	11			3.4
	Onions	6	2	NL		NL		27	2			0.5
		Total: 306			**Total: 84**		**Total: 77**		**Total: 151**	**27.4**	**25.0**	**49.5**

The West region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

[1]Cotton acreage based on 2010 planting data (USDA-NASS, 2011e).

[2]Column C is obtained by multiplying Column A by Column D.

[3]The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist and Extension Weed Control Specialist in cotton ([redacted] University of Arizona; and [redacted] University of California, Personal Communications, November, 2010).

[4]Dicamba usage data in Column E except for cotton and soybean is based on 2010 data (Monsanto Company, 2011). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

[5]Glufosinate usage data in Column G except for cotton is based on 2010 (Monsanto Company, 2011). Glufosinate usage in cotton (50%) is the future market adoption estimate.

[6]Glyphosate usage data in Column I is based on 2010 data (Monsanto Company, 2011).

[7]Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

[8]Newly seeded alfalfa; Glyphosate usage in alfalfa (50%) is future market adoption estimate.

[9]AZ acreage: cauliflower (3), lettuce (62), broccoli (7); U.S. acreage: cauliflower (35), lettuce (199), broccoli (125).

[10]Since no data was reported or the survey sample size was too small for the herbicide data in the state to be statistically reliable, the percent usage for the herbicide/crop in the U.S. was used.

[11]Totals may not be exact due to rounding.

VIII.H.1. Cotton Volunteer Management

Volunteer cotton refers to plants that have germinated, emerged and established unintentionally from the previous year's cotton crop ([redacted] et al., 2002). Volunteer cotton plants generally come from seed that falls to the ground as a result of preharvest losses due to adverse weather condition or losses during the harvesting operation. Volunteer cotton will compete with the rotational crop and potentially cause yield loss and act as early host plants for pests such as spider mites and aphids ([redacted] et al., 2002). Although volunteer cotton in soybean fields can impact yield, recent studies indicate that other common grasses or broadleaf weeds are more problematic in soybean (Lee et al., 2009). The occurrence of volunteer cotton depends on the tillage after harvesting the crop and the severity of winters. Cotton volunteers are more frequently observed in conservation tillage systems where tillage is not used prior to planting. An integrated weed management system of tillage and herbicides has traditionally been the most common method of volunteer cotton control ([redacted] et al., 2002).

Mechanical tillage prior to planting is an effective and efficient method for controlling seedling volunteer cotton plants. This is accomplished in most soil conditions because the root and hypocotyls of seedling cotton are easily destroyed by the tillage process ([redacted] et al., 2002). Any damage occurring below the cotyledons will kill the plant because there are no growing points from which the plant can recover. The disadvantages of tillage are moisture loss under arid conditions and the possibility of increased soil erosion. In-crop cultivation is a highly effective option for satisfactory control of volunteer seedlings. Where cultivation is not appropriate, the use of herbicides is effective in controlling volunteers.

University weed specialists have identified numerous effective and economical herbicide treatments for control of volunteer cotton in the various rotational crops including cotton (Table VIII-25). University studies have shown that the timing of the herbicide application can greatly impact the effectiveness of many herbicides. Newly emerged cotton (up to 2- 3-leaf stage) as a volunteer is much easier to control with herbicides than more mature cotton ([redacted] and [redacted], 2010). If the volunteer cotton plants contain the glyphosate-tolerance trait, the use of glyphosate alone in subsequent rotational crops will not control these seedlings. Similarly, volunteer cotton plants containing the dicamba and glufosinate-tolerant traits from MON 88701 would not be controlled with either dicamba or glufosinate herbicides, and alternative herbicides would be required for control.

Currently both dicamba and glufosinate are labeled for use in crops rotated with cotton. Dicamba herbicide is labeled for weed control in soybean, corn, cotton, sorghum, wheat, barley, oats, millet, pasture, rangeland, asparagus, sugarcane, turf, grass grown for seed, conservation reserve programs, and fallow croplands. Glufosinate is used for postemergence weed control in canola, corn, cotton, and soybean varieties containing glufosinate-tolerance. It may also be applied as a preplant burndown application in conventional or herbicide-tolerant varieties of canola, corn, cotton, soybean, or sugarbeet. The herbicide control options available in rotational crops will continue to result in the ability to manage cotton volunteers.

Preplant burndown applications of carfentrazone, paraquat, or flumioxazin will effectively control emerged volunteer cotton prior to planting rotational crops ([redacted] et al., 2002; [redacted] et al., 2002). In most situations, these preplant measures are sufficient and no additional control measures specifically for cotton volunteers are required the remainder of the season. In the event these measures are not sufficient, the preplant treatment will generally reduce infestation levels, allowing for more effective in-crop management of the remaining volunteer cotton in rotational crops.

In emerged cotton, in-crop cultivation has been traditionally used in the subsequent cotton crop or other crops to effectively remove weeds and volunteer cotton plants between the crop rows. In reduced tillage situations, special high-residue cultivators with sweeps may be used to effectively lift weeds out of the soil to leave the ground cover undisturbed. Cotton emerging within the row can negatively impact cotton growth and management decisions due to increased plant population and disease susceptibility ([redacted] et al., 2002). However, plants remaining at the end of the season can generally be harvested with the planted population by mechanical picking or stripping. Several herbicides are also available for control of volunteer cotton plants after the emergence of the rotational crop. In emerged cotton, applications of carfentrazone or paraquat with hooded sprayers or other selective equipment will effectively control volunteer plants and other weeds in row middles (Alford et al., 2002; [redacted] et al., 2002). However, special precautions must be taken to ensure that these non-selective herbicides do not contact the cotton crop (Gray et al., 2002). Chlorimuron and imazaquin provide control of volunteer cotton in soybean (Clemmer et al., 2001; York et al., 2004) (Table VIII-25). Volunteer cotton in corn generally is not an issue because of the sensitivity of cotton to a number of commonly used corn herbicides (*e.g.*, atrazine).

Table VIII-25. Herbicides and Application Timing for Control of Volunteer Cotton in Labeled Rotational Crops[1]

Rotation Crop/ Herbicide Product	Rate Product/Acre	Preplant / Preemergent	In-Crop
Cotton			
Gramoxone (paraquat)	32 oz	X	X*
Âim (carfentrazone)	1 oz	X	X*
ET (pyraflufen)	1.5 oz	X	X*
Layby Pro (linuron/diuron)	32 oz		X*
Sharpen (saflufenacil)	1.5-2.0 oz	X	
Corn			
Atrazine	32 oz	X	X
Callisto (mesotrione)	3 fl oz	X	X
Sharpen (saflufenacil)	1.5-2.0 oz	X	
Status (diflufenzopyr/dicamba)	0.75 oz	X	X
2,4-D	32 oz	X	X
Soybean			
Resource (flumiclorac)	8 oz		X
Gramoxone Inteon (paraquat)	32 oz	X	
Sencor + Classic (metribuzin + chlorimuron)		X	
Classic (chlorimuron)	2/3 oz		X
2,4-D	32 oz	X	
Peanuts			
Gramoxone Inteon (paraquat)	32 oz	X	
Classic (chlorimuron)	2/3 oz		X
Sunflower			
Paraquat	32 oz	X	
Sharpen (saflufenacil/)	1.5-2.0 oz	X	
Buctril	1 pt		X
Sorghum			
Gramoxone Inteon (paraquat)	32 oz	X	
Sharpen (saflufenacil)	1.5-2.0 oz	X	
Atrazine	32 oz	X	X
Wheat			
CleanWave (aminopyralid/fluroxypyr)	14 oz		X
Buctril (bromoxynil)	1 pt		X
2,4-D	32 oz		X
Starane (fluroxypyr)	16 oz		X
Sharpen (saflufenacil)	1.5-2.0 oz	X	

[1] (Grichar et al., 2010; Keeling et al., 2009; [redacted] et al., 2002; [redacted] and [redacted], 2010; York et al., 2004).

*Hooded or selective equipment only

VIII.I. Stewardship of MON 88701

Monsanto develops effective products and technologies and is committed to assuring that its products and technologies are safe and environmentally responsible. Monsanto demonstrates this commitment by implementing product stewardship processes throughout the lifecycle of a product and by participation in the Excellence Through Stewardship[SM] (ETS) Program (BIO, 2010). These policies and practices include rigorous field compliance and quality management systems and verification through auditing. Monsanto's Stewardship Principles are also articulated in Technology Use Guides (Monsanto Company, 2012) and Monsanto Technology Stewardship Agreements that are signed by growers who utilize Monsanto branded traits, to ensure stewardship compliance.

As an integral action of fulfilling this stewardship commitment, Monsanto will seek biotechnology regulatory approvals for MON 88701 in all key cotton import countries with a functioning regulatory system to assure global compliance and support the flow of international trade. These actions will be consistent with the Biotechnology Industry Organization (BIO) Policy on Product Launch (BIO, 2010). Monsanto continues to monitor other countries that are key importers of cotton 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. In addition, Monsanto actively interacts with and participates in cotton industry groups, such as the National Cotton Council, state grower boards, Farm Bureau, Cotton Inc., and trade affiliates, to obtain input on market trends to ensure awareness of the current key markets for whole cottonseed and cottonseed by-products.

Monsanto also commits to industry best practices on seed quality assurance and control to ensure the purity and integrity of MON 88701 cottonseed. As with all of Monsanto's products, before commercializing MON 88701 in any country, a MON 88701 detection method will be made available to cotton producers, processors, and buyers.

The dicamba and glufosinate-tolerant cotton system, which is applying dicamba and/or glufosinate herbicide to MON 88701 integrated into the glyphosate cotton systems, will enable expanded use of dicamba herbicide in cotton production. Monsanto is seeking regulatory approvals with the U.S. EPA for the expanded application of dicamba herbicide as a weed control tool in cotton. Furthermore, Monsanto will establish appropriate dicamba Maximum Residue Levels (MRLs) for key cotton import countries. No additional regulatory approvals with U.S. EPA will be required for glufosinate products for use in MON 88701.

As with all U.S. EPA registered herbicides for agricultural use, it is possible that offsite movement during and/or following application can occur such that non-target plants may be exposed to direct spray or to spray drift. Research has demonstrated that herbicide formulation, application equipment, and application procedures can be optimized to significantly reduce spray drift potential in most circumstances (Jordan et al., 2009; SDTF, 1997). Monsanto is addressing potential offsite movement of dicamba by seeking U.S. EPA registration of a low volatility dicamba formulation (DGA salt) for ground

application only. Additionally Monsanto will implement a robust stewardship program that will include a strong emphasis on grower and applicator training. Furthermore, Monsanto will consult with U.S. EPA to identify what additional measures, if any are necessary, to address any potential impact of off-site movement of these herbicides.

Stewardship of dicamba and glufosinate, to preserve their usefulness for growers, is also an important aspect of Monsanto's stewardship commitment. Detailed information regarding dicamba and glufosinate weed resistance and the usefulness of dicamba and glufosinate-tolerant cotton in combination with glyphosate-tolerant cotton to address herbicide-resistance issues is presented in Section VIII.G and Appendix I.

VIII.J. Impact of the Introduction of MON 88701 on Agricultural Practices

Introduction of MON 88701 is expected to have no impact on current agronomic, cultivation and management practices for cotton, with the exception of expanded dicamba application timings and more options for effective weed management. Dicamba has been used in corn, soybean, and small grain cropping systems since 1967. MON 88701 with its excellent crop tolerance to dicamba allows preemergence applications through crop emergence and in-crop postemergence applications up to seven days prior to harvest. MON 88701 will be combined with glyphosate-tolerant cotton systems utilizing traditional breeding techniques. Cotton containing both MON 88701 and glyphosate-tolerance will allow the use of glyphosate, dicamba, and glufosinate herbicides in an integrated weed management program to control a broad spectrum of grasses and broadleaf weed species, and to sustain and complement the benefits and value of the glyphosate use in glyphosate-tolerant cotton systems.

MON 88701 has been shown to be comparable to commercially cultivated cotton in its agronomic, phenotypic, and compositional characteristics (refer to Sections VI, VII, and VIII), and has the same levels of susceptibility to insect pests and diseases as commercial cotton. Like other herbicide-tolerant cotton, such as glyphosate-tolerant cotton that have been cultivated and consumed in the U.S. since 1996, dicamba and glufosinate tolerant cotton (MON 88701) will improve the current agricultural practices for U.S. cotton growers by providing two additional in-crop herbicide modes-of-action for the control of glyphosate's hard-to-control and resistant broadleaf weeds, as well as weeds resistant to other herbicide families, thereby improving the efficiency in the U.S. cotton production system to maximize or maintain cotton yield potential, and help meet growing needs for fiber, food, and feed.

IX. ENVIRONMENTAL ANALYSIS

IX.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 88701 and its impact on current agronomic practices. USDA-APHIS has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition must be granted, thereby allowing unrestricted introduction of the article.

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

The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the regulated article is unlikely to pose a plant pest risk. The approach used to assess the plant pest potential of MON 88701 is a weight-of-evidence approach based primarily on eight lines of evidence: 1) insertion of a single functional copy of the *dmo* and *bar* expression cassettes; 2) characterization of MON 88701 DMO and PAT (*bar*) expressed in MON 88701; 3) protein safety of MON 88701 DMO and PAT (*bar*); 4) compositional equivalence of harvested MON 88701 seed to conventional commercial cotton; 5) phenotypic, agronomic, plant mapping, and environmental interaction characteristics demonstrating no increased plant pest potential compared to commercially cultivated cotton, including disease and pest susceptibilities; 6) negligible risk to NTOs; 7) familiarity with cotton as a cultivated crop and the inherently low plant pest potential of cotton; and 8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds (other than the intended benefits of dicamba and glufosinate for weed control), diseases, and insects than commercially cultivated cotton.

Using the aforementioned assessment, the data and analysis presented in this petition lead to a conclusion that MON 88701 is unlikely to be a plant pest, and therefore should no longer be subject to regulation under 7 CFR Part 340.

IX.B. Plant Pest Assessment of MON 88701 Insert and Expressed Proteins

This section summarizes the details of the genetic insert, characteristics of the genetic modification, and safety and expression of the MON 88701 DMO and PAT (*bar*) proteins used to evaluate the food, feed, and environmental safety of MON 88701.

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

IX.B.1.1. Genetic Insert

As described in Section IV, molecular analyses demonstrated that MON 88701 contains a single copy of the inserted T-DNA at a single integration locus. No backbone sequences from the PV–GHHT6997 were detected in the genome of MON 88701. In addition, data confirmed the organization and sequence of the insert and the stability of the insert over several breeding generations.

IX.B.1.2. Mode-of-Action

MON 88701 exhibits tolerance to the herbicide dicamba through the insertion of a demethylase gene from *Stenotrophomonas maltophilia* that encodes DMO and the herbicide glufosinate through the insertion of a N-acetyltransferase gene from *Streptomyces hygroscopicus* that encodes PAT. The DMO protein is a Rieske-type non-heme iron oxygenase that catalyzes the demethylation of dicamba to the non-herbicidal compound DCSA (Section V.A.1.). As shown in section V.A. and by Dumitru et. al. (2009), DMO is specific for dicamba.

The PAT protein has been extensively assessed, as numerous glufosinate-tolerant products including those in cotton, corn, soy, canola, sugarbeet and rice have been reviewed by the USDA and several other regulatory agencies (ILSI-CERA, 2011; OECD, 1999b; 2002a). The PAT (*bar*) protein produced in MON 88701 acetylates the free amine group of L-phosphinothricin form of glufosinate to produce non-herbicidal N-acetyl glufosinate (Section V.A.2.). PAT is specific for glufosinate ([redacted] et al., 1987; Wehrmann et al., 1996).

IX.B.1.3. Protein Safety and Expression

The safety of the expressed proteins is detailed in Section V. A history of safe use has been established for both MON 88701 DMO and PAT (*bar*) proteins (Section V.E). MON 88701 DMO and PAT (*bar*) lack structural similarity to known allergens, gliadins, glutenins, or protein toxins (Section V.D). MON 88701 DMO and PAT (*bar*) are present at very low levels in MON 88701 cottonseed (Section V.C.) and will constitute a small portion of the total protein present in feed derived from MON 88701 (Section V.E.). No consumption of the MON 88701 DMO or PAT (*bar*) proteins derived from MON 88701 is expected for the U.S. general population at the present time given that the only foods produced from cottonseed are RBD oil and linters, which contain undectable and neligible amounts of total protein, respectively. As shown in Section V.E, MON 88701 DMO and PAT (*bar*) are readily digestible in simulated gastric and simulated intestinal fluids and show no oral toxicity in mice (Section V.E.). In addition, PAT proteins have been evaluated in several previous safety assessments with no safety concerns identified.

IX.B.2. Compositional Characteristics

Detailed compositional analyses in accordance with OECD guidelines were conducted to assess whether levels of key nutrients and anti-nutrients in MON 88701, both treated and not treated with dicamba or glufosinate herbicides, were comparable to levels present in the near isogenic conventional cotton control Coker 130 and several conventional, commercial reference varieties (Section VI). Seed were harvested from eight individual sites in which MON 88701, the conventional control, and a range of commercial reference varieties were grown concurrently in the same field trial. The commercial reference varieties were used to establish a range of natural variability for the key nutrients and anti-nutrients in commercial cotton varieties that have a history of safe consumption.

The combined-site analysis was conducted to determine statistically significant differences (5% level of significance) between MON 88701 treated with dicamba and glufosinate and the conventional control. The biological significance of difference from the combined-site data were reviewed using considerations relevant to food and feed safety and nutritional quality. These considerations included: 1) the relative magnitude of differences in the mean values of nutrient and anti-nutrient components of MON 88701 and the conventional control; 2) whether the MON 88701 component mean value was within the range of natural variability of commercial cotton as represented by the 99% tolerance interval of the commercial reference varieties grown concurrently in the same field trial; 3) whether the MON 88701 component mean value was within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database; and 4) analyses of the reproducibility of the statistically significant combined-site component differences at individual sites.

Assessment of the analytical results confirmed that the differences observed in the combined-site analysis were not meaningful to food and feed safety or the nutritional quality of MON 88701 cotton. To further support the safety assessment, similar compositional analyses were also conducted on cottonseed from MON 88701 not treated with dicamba or glufosinate herbicides. Based on the analyzed nutrient and anti-nutrient levels of both dicamba and glufosinate-treated and not treated MON 88701, MON 88701 is compositionally equivalent to conventional commercial cotton and therefore the food and feed safety and nutritional quality of this product is comparable to that of commercially cultivated cotton. These results support the overall conclusion that MON 88701 is unlikely to be a plant pest.

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

An extensive set of comparative plant characterization data were used to assess whether the introduction of the dicamba and glufosinate tolerance traits altered the plant pest potential of MON 88701 compared to the conventional control (Section VII). Phenotypic, agronomic, plant mapping, and environmental interaction characteristics of MON 88701 were evaluated and compared to those of the conventional control (Section

VII.B). As described below, these assessments included: seed dormancy and germination characteristics; agronomic and phenotypic characteristics; plant mapping characteristics; observations for abiotic stress response, disease damage, arthropod-related damage, arthropod abundance, and pollen characteristics. To support the trait assessment, similar observations were also conducted on MON 88701 treated with dicamba and glufosinate herbicides. Results from all phenotypic, agronomic, plant mapping, and environmental interaction assessments demonstrated that MON 88701 does not possess weedy characteristics, or increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors compared to the conventional control. Taken together, the results of the analysis support a determination that MON 88701 is no more likely to pose a plant pest risk or have a biologically meaningful change in environmental impact than commercially cultivated cotton.

IX.B.3.1. Seed Dormancy and Germination

Seed dormancy and germination characterization demonstrated that MON 88701 seed had germination characteristics similar to those of the conventional control (Section VII.C.1). In particular, the lack of hard seed, a well-accepted characteristic often associated with plants that are weeds, supports a conclusion of no increased weediness or plant pest potential of MON 88701 compared to commercially cultivated cotton.

IX.B.3.2. Plant Growth and Development

Of the growth and development characteristics assessed between MON 88701 and the conventional control, eight significant differences were detected in a combined-site analysis (Section VII.C.2.1). MON 88701 observed a reduction in plant height at 30 DAP and at harvest, increased number of nodes above white flower at observations 2 and 3, a lower seed index, increased total and immature seed per boll, and increased fiber strength. The differences in mean values of MON 88701 were within the range of the commercial reference varieties for these characteristics. Thus, the differences in these parameters are not considered to be biologically meaningful in terms of increased weediness or plant pest potential of MON 88701 compared to commercially cultivated cotton.

Plant mapping is a process commonly used to quantify growth and development parameters of the cotton plant, including boll retention and distribution (Section VII.C.2.2). Of the plant mapping characteristics assessed between MON 88701 and the conventional control, one significant difference was detected where MON 88701 had increased first-position bolls per plant compared to the conventional control. However, the mean value of the number of first-position bolls for MON 88701 was within the range of values observed for the commercial reference varieties. Thus, it is unlikely that the difference is biologically meaningful in the context of increased weediness of MON 88701 compared to commercially cultivated cotton.

IX.B.3.3. Response to Abiotic Stressors

No biologically meaningful differences were observed during comparative field observations between MON 88701 and the conventional control in their response to abiotic stressors, such as compaction, drought, high winds, nutrient deficiency, etc. (Section VII.C.2.3). The lack of significant biologically meaningful differences in the MON 88701 response to abiotic stress support the conclusion that the introduction of the dicamba and glufosinate-tolerance traits are unlikely to result in increased weediness or plant pest potential compared to commercially cultivated cotton.

IX.B.3.4. Pollen Morphology and Viability

Evaluations of pollen morphology and viability from field-grown plants provide information useful in a plant pest assessment as it relates to the potential for gene flow and introgression of the biotechnology-derived trait(s) into other cotton varieties and wild relatives (Section VII.C.3). Pollen morphology and viability evaluations demonstrated no statistically significant differences between MON 88701 and the conventional control. Taken together, these comparative assessments indicate that MON 88701 is not likely to have increased weediness or plant pest potential compared to commercially cultivated cotton.

IX.B.3.5. Interactions with Non-target Organisms

Evaluation of MON 88701 for potential adverse impacts on NTOs is a component of the plant pest risk assessment. Since MON 88701 is not intended to have pesticidal activity, all organisms that interact with MON 88701 can be considered to be NTOs. In 2010 U.S. field trials, observational data on environmental interactions were collected for MON 88701 and the conventional control. In addition, multiple commercial reference varieties were included in the analysis to establish a range of natural variability for each characteristic among commercial cotton varieties. The environmental interactions assessment (Section VII.C.2.3) included data collected on plant-arthropod, plant-disease interactions, and plant mapping. The results of this assessment indicated that the presence of the dicamba and glufosinate-tolerance traits did not alter plant-arthropod interactions, including beneficial arthropods and arthropod pests, nor did it alter disease susceptibility of MON 88701 compared to conventional cotton. In addition, there were no differences in plant mapping parameters between MON 88701, not treated with dicamba or glufosinate herbicides, and the conventional control that would be indicative of a differential plant response to abiotic or biotic stressors. Thus, since all plots evaluated for plant mapping characteristics were at the same sites they would be subjected to similar stressors. Given that MON 88701 plants treated and not treated with dicamba and glufosinate herbicides had similar plant map results, it can be concluded that both responded to stressors in a similar manner. Therefore, these data support the conclusion that the biotechnology-derived traits in MON 88701, treated or not treated with dicamba and glufosinate herbicides, are unlikely to have increased plant pest potential, weediness, or an adverse environmental impact compared to commercially cultivated cotton. The lack of biologically meaningful differences in disease damage, arthropod-related damage, pest- and beneficial-arthropod abundance, and plant mapping

data demonstrate that the introduction of the dicamba and glufosinate-tolerance traits are unlikely to be biologically meaningful in terms of increased plant pest potential as compared to commercially cultivated cotton.

The potential for MON 88701 to influence NTOs was evaluated using a combination of biochemical information and experimental data. The biochemical information and experimental data included molecular characterization, the MON 88701 DMO and PAT (*bar*) safety assessments, the history of environmental exposure to mono-oxygenases (the class of enzymes to which DMO belongs) and PAT proteins, results from the environmental interactions assessment described above, and the demonstration of compositional, agronomic and phenotypic equivalence to conventional cotton. Taken together, these data support the conclusion that MON 88701 is unlikely to adversely affect NTOs, including those beneficial to agriculture. Any effects on non-target organisms that could potentially result from proposed changes in herbicide labels will be evaluated by the EPA.

IX.C. Weed Potential of MON 88701

Cotton is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1997), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). United States Department of Agriculture has previously determined that "cotton is not considered to be a serious, principal or common weed pest in the U.S." (USDA-APHIS, 1995). Commercial *Gossypium* species in the U.S. are not considered weeds and are not effective in invading established ecosystems. Cotton is not considered to have weedy characteristics in the U.S. and does not possess attributes commonly associated with weeds, such as long soil persistence, the ability to invade and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. It is recognized that in some agricultural systems, cotton can volunteer in a subsequent rotational crop. However, volunteers are easily controlled through tillage or use of appropriate herbicides (Alford et al., 2002; [redacted] et al., 2002; [redacted] et al., 2002).

In comparative studies between MON 88701 and the conventional control, phenotypic, agronomic, plant mapping, and environmental interaction data were evaluated (Section VII) for changes that would impact the plant pest potential and in particular, plant weed potential. Results of these evaluations show that there is no biologically meaningful difference between MON 88701 and the conventional control for characteristics potentially associated with weediness. Furthermore, comparative field observations between MON 88701 and its conventional control in their response to abiotic stressors, such as drought, heat stress, and high winds, indicated no biologically meaningful differences and, therefore, no increased weed potential. Data on environmental interactions also indicate that MON 88701 does not confer any biologically meaningful increased susceptibility or tolerance to specific diseases or insect pests. Collectively, these findings support the conclusion that MON 88701 has no increased weediness compared to commercially cultivated cotton.

Volunteer MON 88701, like volunteer commercial cotton, would compete poorly with any succeeding crops and soon die, making it extremely unlikely to have any prolonged negative effects. Volunteer MON 88701 would also not be "extremely difficult to manage" because it can be controlled easily with numerous alternative herbicides and other mechanical means (Alford et al., 2002; [redacted] et al., 2002; [redacted] et al., 2002).

IX.D. Potential for Pollen Mediated Gene Flow and Introgression

Pollen mediated gene flow (often referred to as cross pollination) occurs when pollen of one plant fertilizes ovules of a second plant. Pollen mediated gene flow is affected by both biotic and abiotic factors such as plant biology, pollen biology/volume, plant phenology, overlap of flowering times, proximity of the pollen source and sink, ambient conditions such as temperature and humidity, and field architecture. Pollen mediated gene flow is a natural biological process, and therefore does not constitute an environmental risk in and of itself.

Introgression is a process whereby one or more genes successfully incorporate into the genome of a recipient plant. Pollen mediated gene flow and 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 and plant populations will result in increased plant pest potential. The potential for gene flow and introgression from deregulation of MON 88701 is discussed in greater detail below.

IX.D.1. Hybridization with Cultivated Cotton

Although natural crossing can occur, cotton is normally considered to be a self-pollinating crop (Niles and Feaster, 1984). There are no morphological barriers to cross-pollination based on flower structure. However, the pollen is heavy and sticky and transfer by wind is limited. Pollen is transferred instead by insects, in particular by various wild bees, bumble bees (*Bombus* sp.), and honeybees (*Apis mellifera*) (Van Deynze et al., 2005). Numerous studies on cotton cross-pollination have been conducted, and the published results, with and without supplemental pollinators, are summarized in Table IX-1. Recent cotton literature shows that the frequency of cross-pollination decreases with distance from the pollen source. [redacted] (1976) traced movement of pollen by means of fluorescent particles and found that, even among flowers located only 150 to 200 feet from a cotton field that was surrounded by a large number of bee colonies to ensure ample opportunity for transfer of pollen, fluorescent particles were detected on only 1.6% of the flowers. In a 1996 study with various field designs, Llewellyn and Fitt (1996) also found low levels of cross-pollination in cotton. At one meter from the source they observed cross-pollination frequencies of 0.15 to 0.4%, decreasing to below 0.3% at 16 meters from the source. Umbeck et al. (1991) used a selectable marker to examine cross-pollination from a 30 x 136 meter source of biotechnology-derived cotton. Cross-pollination decreased from five to less than one percent from one to seven meters, respectively, away from the source plot. A low level of cross-pollination (less than one percent) was sporadically detected at the furthest sampling distance of 25 meters. Berkey et al. (2002) reported that cross pollination between fields separated by a 13 foot road

decreased from 1.89% in the row nearest the source to zero percent in the 24th row. Van Deynze et al. (2005) conducted a two year study on pollen-mediated gene flow with high and low pollinator activity. In the presence of high pollinator activity the pollination frequency was 7.65% at 0.3 meters and less than 1% at greater than nine meters. Whereas, the pollination frequency in the presence of low pollinator activity was below 1% at just over a meter. In a 2008 study, pollination frequencies of 5.00% and 0.00% were demonstrated at 1 and 8 meters, respectively (Kairichi et al., 2008).

The potential for outcrossing and gene introgression from MON 88701 to cultivated cotton in the U.S. is low since cotton pollen movement by wind is limited due to it is large and sticky nature, and several studies have demonstrated that cross-pollination, even in the presence of high pollinator activity, is limited by distance. Therefore, the environmental consequences of pollen transfer from MON 88701 to other cotton or related *Gossypium* species is considered to be negligible.

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

Distance from Pollen Source (meters)	Cross-Pollination (%)	Comments	Reference
45-61	1.60%	Used fluorescent particles to follow pollinator movement in cotton fields over one season.	([redacted], 1976)
1 4 16	0.15-0.4% <0.08% <0.03%	Used a selectable marker to examine cross-pollination in the progeny of buffer row plants over one season.	(Llewellyn and Fitt, 1996)
1 1-25	5% <1%	Used a selectable marker to examine cross-pollination from a 20 x 136 meter source of biotechnology-derived cotton over one season.	(Umbeck et al., 1991)
5 10.5 17 25	1.89% 0.77% 0.13% 0.00%	Used herbicide bioefficacy to examine pollen flow between fields separated by a 13 foot road over one season.	(Berkey et al., 2002)
0.3 >9 >1 1625	7.65% * < 1% * < 1% ** 0.04% **	Used herbicide bioefficacy confirmed by DNA testing to measured pollen-mediated gene flowing in four directions over 2 years.	(Van Deynze et al., 2005)
1 2-7 8	5.00% 2.00% 0.00%	Used ELISA strips to examine pollen-mediated gene flow in four directions from Bt source over a period of one season.	(Kairichi et al., 2008)

* High pollinator activity
** Low pollinator activity

IX.D.2. Hybridization with Wild and Feral *Gossypium* species

Based on cytological evidence, seven genomic types, A through G, many with subtypes, have been identified for the genus *Gossypium* (Endrizzi et al., 1984). The domesticated species *G. hirsutum* and *G. barbadense* are allotetraploid (AADD, 2n=4x=52), while *G. thurberi* is a diploid (DD, 2n=2x=26), and *G. tomentosum* is an allotetraploid (AADD, 2n=4x=52). *G. tomentosum* is capable of crossing with domesticated cotton to produce fertile offspring (Waghmare et al., 2005). However, Hawaii is the only U.S. region where *G. tomentosum* is found and domesticated cotton is not grown commercially in Hawaii, with the exception of potential counter-season breeding nurseries where appropriate isolation distances and practices are required (Wagner et al., 1990). Thus, the potential for gene flow to these wild relatives is limited. Importantly, MON 88701 would not be expected to confer a selective advantage to, or enhance the pest potential of, progeny resulting from such a cross if it were to occur. Any potential gene exchange between *G. thurberi* and domesticated cotton, if it were to occur, would result in triploid

(ADD, 3x=39), sterile plants because *G. hirsutum* and *G. barbadense* are allotetraploids (AADD, 2n=4x=52) and *G. thurberi* is a diploid (DD, 2n=2x=26). Such sterile hybrids have not been observed to persist in the wild. Fertile allohexaploids (6x=78) have not been reported in the wild.

Only two 'wild' *Gossypium* species related to cultivated cotton are known to be present in the U.S., *G. thurberi* Todaro, which is known in Arizona (Fryxell, 1984), and feral populations of cultivated *G. hirsutum* and 'wild' populations of *G. hirsutum* are known to occur in South Florida and Puerto Rico (Brubaker et al., 1999). Both of these species would be capable of crossing with cultivated cotton, but they are not known to exist in cotton growing areas. Importantly, MON 88701 would not be expected to confer a selective advantage to, or enhance the pest potential of, progeny resulting from such crosses if they were to occur.

Importantly, the environmental consequences of pollen transfer from MON 88701 to other cotton or related *Gossypium* species is considered to be negligible due to the plant biology and limited movement of cotton pollen, the safety of the introduced protein, and the lack of any selective advantage by the dicamba and glufosinate traits that might be conferred on a recipient plant of feral or wild cotton, or a wild relative.

IX.D.3. Transfer of Genetic Information to Species with which Cotton Cannot Interbreed (Horizontal Gene Flow)

Monsanto is unaware of any reports regarding the unaided transfer of genetic material from cotton species to other sexually-incompatible plant species. The likelihood for horizontal gene flow to occur is exceedingly small. Therefore, potential ecological risk associated with horizontal gene flow from MON 88701 due to the presence of the dicamba and glufosinate-tolerance traits are not expected. The consequence of horizontal gene flow of the dicamba and glufosinate-tolerance traits into other plants that are sexually-incompatible is negligible since, as data presented in this petition confirm, the genes and traits confer no increased plant pest potential to cotton. Thus, in the highly unlikely event that horizontal gene transfer were to occur, the presence of the dicamba and glufosinate-tolerance traits would not be expected to increase pest potential in the recipient species.

IX.E. Potential Impact on Cotton Agronomic Practices

An assessment of current cotton agronomic practices was conducted to determine whether the cultivation of MON 88701 has the potential to impact current cotton and weed management practices (Section VIII). Cotton fields are typically highly managed agricultural areas that are dedicated to crop production. MON 88701 is likely to be used in common rotations on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed containing MON 88701 to growers. Cultivation of MON 88701 is not expected to differ from typical cotton cultivation, with the exception of an expanded window of dicamba applications. As glufosinate is already utilized within U.S. cotton growing areas, no change in agronomic practices or land use would occur with the cultivation of

MON 88701 and the presence of the glufosinate-tolerance trait. Due to the crop safety of MON 88701 to dicamba and glufosinate, growers will have two herbicide modes-of-action for in-crop control of glyphosate's hard-to-control and resistant broadleaf weeds that are present in U.S. cotton production. As a result of cultivation of MON 88701 integrated into the glyphosate-tolerant cotton systems, the number of dicamba-treated cotton acres will likely increase, whereas the number of glufosinate-treated cotton acres is expected to remain relatively static with minimal increase in use as cotton varieties utilizing the biotechnology-derived glufosinate-tolerance trait are currently commercially available and being utilized across the U.S. cottonbelt. Additionally, due to the expanded timing of in-crop applications to cotton, dicamba treatments will be later in the growing season than most current labeled dicamba uses.

MON 88701 is similar to commercially cultivated cotton in its agronomic, phenotypic, ecological, and compositional characteristics, and has levels of resistance to insect pests and diseases comparable to commercially cultivated cotton. Based on this assessment, the introduction of MON 88701 is not likely to impact current U.S. cotton agronomic or cultivation practices, or weed management practices, other than the intended weed control benefits.

IX.F. Conventional Breeding with Other Biotechnology-derived or Conventional Cotton

Several biotechnology-derived cotton products have been deregulated or are under consideration for deregulation. Once deregulated, MON 88701 may be bred with these deregulated biotechnology-derived cotton products, as well as with conventional cotton, creating new improved varieties. APHIS has determined that none of the individual biotechnology-derived cotton products it has previously deregulated displays increased plant pest characteristics. APHIS has also concluded that any progeny derived from crosses of these deregulated biotechnology-derived cotton products with conventional or previously deregulated biotechnology-derived cotton are unlikely to exhibit new plant pest properties. This presumption, that combined-trait biotechnology products are unlikely to exhibit new characteristics that would pose new plant pest risks or potential environmental impacts not observed in the single event biotech product, is based upon several facts. Namely: 1) stability of the genetic inserts is confirmed in each approved biotech-derived cotton product across multiple generations (See Section IV.E for MON 88701 data); 2) stability of each of the introduced traits is continually and repeatedly assessed as new combined-trait varieties are created by plant breeders and tested over multiple seasons prior to commercialization; 3) combined-trait products are developed using the well established process of conventional breeding that has been safely used for thousands of years to generate new varieties (Cellini et al., 2004; NRC, 2004; WHO, 1995); 4) worldwide organizations, such as World Health Organization, Food and Agriculture Organization/World Health Organization, International Seed Federation, CropLife International and U.S. FDA, conclude that the safety of the combined-trait product can be based on the safety of the parental GE events (CLI, 2005; FAO-WHO, 1996; ISF, 2005; U.S. FDA, 2001; WHO, 1995); and 5) practical applications in the field have shown that two unrelated biotechnology traits combined together by conventional breeding do not display new characteristics or properties

distinct from those present in the single event biotech products (Brookes and Barfoot, 2012; [redacted], 2010; Lemaux, 2008; Pilacinski et al., 2011; Sankula, 2006).

Therefore, based on the considerations above and the conclusion that MON 88701 is no more likely to pose a plant pest risk than commercially cultivated cotton it can be concluded that any progeny derived from crosses between MON 88701 and conventional cotton or deregulated biotechnology-derived cotton are no more likely to pose a plant risk than commercially cultivated cotton.

IX.G. Summary of Plant Pest Assessments

Plant pests are defined in the PPA as certain living organisms that can directly or indirectly injure, cause damage to, or cause disease to any plant or plant product (7 U.S.C. § 7702[14]). Characterization data presented in Sections III through VII of this petition confirm that although MON 88701 contains the dicamba and glufosinate-tolerant traits, it is not different from commercially cultivated cotton in terms of pest potential in its phenotypic, agronomic, plant mapping, and environmental interaction characteristics. Monsanto is not aware of any study results or observations associated with MON 88701 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 88701 compared to commercially cultivated cotton, followed by a risk assessment on detected differences. The risk assessment considered various factors, including: 1) insertion of a single functional copy of the *dmo* and *bar* expression cassettes; 2) characterization and safety of the MON 88701 DMO and PAT (*bar*) proteins; 3) compositional equivalence of harvested MON 88701 cottonseed as compared to commercially cultivated cotton; 4) phenotypic, agronomic, and environmental interaction characteristics demonstrating no increased plant pest potential compared to commercially cultivated cotton; 5) negligible risk to NTOs; 6) familiarity with cotton as a cultivated crop and the inherently low plant pest potential of cotton; and 7) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects than commercially cultivated cotton, with the exception of the expanded window of dicamba application.

Based on the data and information presented in this petition, it is concluded that, like conventional cotton and currently deregulated biotechnology-derived cotton, MON 88701 is highly unlikely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 88701 and any progeny derived from crosses between MON 88701 and other commercial cotton be granted nonregulated status under 7 CFR Part 340.

X. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto does not know of any results or observations associated with MON 88701 or the MON 88701 DMO and PAT (*bar*) proteins indicating that there would be an adverse environmental consequence from the introduction of MON 88701. MON 88701 contains DMO and PAT that confers dicamba and glufosinate tolerance to the cotton plant, respectively. As demonstrated by field results and laboratory tests, the only phenotypic differences between MON 88701 and conventional cotton are the tolerances to dicamba and glufosinate herbicides.

The data and information presented in this petition demonstrate that MON 88701 is unlikely to pose an increased plant pest risk or to have an adverse environmental consequence compared commercially cultivated cotton. This conclusion is reached based on multiple lines of evidence developed from a detailed characterization of the product compared to commercially cultivated cotton, followed by a risk assessment on detected differences. The characterization evaluation included molecular analyses, which confirmed the insertion of a single functional copy of the *dmo* and *bar* expression cassettes at a single locus within the cotton genome. The amino acid sequence of the MON 88701 DMO and PAT (*bar*) proteins expressed in MON 88701 are identical to the amino acid sequences of the respective *E. coli*-produced proteins utilized in the protein safety studies supporting the safety of the proteins. Analyses of key nutrients and, anti-nutrients of MON 88701 seed demonstrate that MON 88701 is compositionally equivalent to commercially cultivated cotton. The phenotypic evaluations of MON 88701, including an assessment of seed germination and dormancy characteristics, plant growth and development characteristics, plant mapping parameters, pollen characteristics, and environmental interactions also indicated that MON 88701 is no more weedy than commercially cultivated cotton. There is no indication that MON 88701 would have an adverse impact on beneficial or non-target organisms. Therefore, based on the lack of increased plant pest potential or adverse environmental consequences compared to commercially cultivated cotton, the risks for humans, animals, and other NTOs from introducing MON 88701 are negligible under the conditions of use.

Successful integration of MON 88701 into glyphosate-tolerant cotton systems will provide growers with an opportunity for an efficient, effective weed management system for the management of glyphosate's hard-to-control and resistant broadleaf weeds; provide a flexible system for inclusion of a second and third herbicide mode-of-action in cotton production practices as recommended by weed science experts to manage weed resistance development; and continue to provide cotton growers with effective weed control systems necessary for production yields to meet the growing needs of the food, feed, and industrial markets.

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APPENDICES

Appendix A: Notifications

Field trials of MON 88701 have been conducted in the U.S. since 2007. The protocols for these trials include field performance, breeding and observation, agronomics, and generation of field materials and data necessary for this petition. In addition to the MON 88701 phenotypic assessment data, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2011-2012 seasons, are still in preparation. A list of trials conducted under USDA notifications and the status of the final reports for these trials are provided in Table A-1.

Table A-1. USDA Notifications and Permits Approved for MON 88701 and Status of Trials Conducted under These Notifications

USDA #	Effective Date	# Release sites per state	Trial Status
2007			
07-241-107n	9/28/2007	PR-2	Submitted to USDA
2008			
08-042-109n	3/12/2008	TX-2, TN-1, NC-2, MS-3, GA-4	Submitted to USDA
08-056-112n	3/26/2008	NM-2	Submitted to USDA
08-056-117n	3/26/2008	TX-3, SC-2, NC-2, MS-2, LA-1, GA-4, AR-1	Submitted to USDA
08-266-130n	10/19/2008	PR-3	Submitted to USDA
2009			
09-058-104n	3/29/2009	CA-1	Submitted to USDA
09-065-111n	4/5/2009	AZ-5, GA-1, MS-3, SC-2, TX-4	Submitted to USDA
09-068-108n	4/8/2009	AL-1, AR-2, AZ-1, GA-1, IL-1, LA-1, MS-1, NC-4, NM-2, TX-1	Submitted to USDA
09-072-103n	4/8/2009	AR-1, MS-2, SC-5, TN-2, TX-5	Submitted to USDA
09-224-101n	9/21/2009	PR-2	Submitted to USDA
2010			
10-054-134n	3/20/2010	TX-4	Submitted to USDA
10-059-109n	3/28/2010	GA-2, NC-9, SC-3	Submitted to USDA
10-061-102n	7/10/2010	MS-1, PR-7	Submitted to USDA
10-064-101n	4/3/2010	CA-2, GA-1, LA-1, MO-1, OK-3, SC-1, AR-1	Submitted to USDA
10-067-104n	4/7/2010	AZ-5, IL-1, MS-4, NM-2, PR-2, TX-10	Submitted to USDA
10-071-101n	4/9/2010	AR-4, AZ-2, GA-2, KS-1, LA-1, NC-2, NM-1, SC-1, TX-2	Submitted to USDA
10-071-102n	4/10/2010	AR-1, GA-1, LA-1, MS-1, NC-1, SC-1, TN-1, TX-2	Submitted to USDA
10-242-102n	9/29/2010	PR-2	Submitted to USDA
10-285-105n	11/11/2010	AR-1, GA-1, LA-1, NM-1	Submitted to USDA
2011			
11-045-101n	3/16/2011	MS-1, PR-2	Pending
11-052-105n	3/23/2011	AL-1, FL-2, GA-9, MS-1, NC-6, SC-4	Pending
11-053-105n	3/25/2011	AR-3, LA-2, MO-2, MS-8, TN-5, TX-4	Pending
11-075-107n	4/15/2011	AL-1, AR-1, AZ-4, IL-1, LA-1, MO-1, MS-4, NC-1, SC-1, TX-9	Pending
11-068-103n	4/8/2011	AL-2, AR-2, AZ-1, CA-2. GA-2, LA-1, NC-1, NM-1, SC-1, TX-5	Pending
11-083-104n	4/23/2011	AL-1,MS-1	Pending
11-084-107n	4/24/2011	NC-1	Pending
11-091-102n	5/1/2011	TX-1	Pending

Table A-1. USDA Notifications and Permits Approved for MON 88701 and Status of Trials Conducted under These Notifications (continued)

USDA #	Effective Date	# Release sites per state	Trial Status
2011 cont.			
11-094-101n	5/4/2011	AZ-1	Pending
11-111-104n	5/21/2011	FL-1	Pending
11-133-103n	6/12/2011	IL-1	Pending
11-153-101n	7/2/2011	MS-1, PR-2	Pending
11-152-101n	7/1/2011	GA-1	Pending
11-199-102n	8/17/2011	PR-1	Pending
11-290-101n	11/16/2011	MS-1, PR-3	Pending
2012			
12-018-101n	2/17/2012	AL-1, TX-2	Pending
12-053-110n	3/23/2012	AR-3, CA-1, GA-2, LA-2, MS-11, NC-1, TN-1, TX-2	Pending
12-046-104n	3/16/2012	AL-1, AR-4, FL-1, GA-2, LA-1, NC-3, SC-1, TN-4, TX-2	Pending
12-051-106n	3/21/2012	AL-3, AR-3, FL-1, GA-3, MS-7, SC-1, TN-1, TX-5	Pending
12-051-105n	3/21/2012	GA-5, MS-4, NC-6, SC-2, TN-1, TX-5	Pending
12-046-109n	3/16/2012	AR-1, MO-5, TN-13, TX-2	Pending
12-055-101n	3/25/2012	AR-1, CA-1, SC-1, TX-1	Pending
12-068-101n	4/7/2012	CA-4	Pending
12-053-109n	3/23/2012	AL-1, NC-1, SC-1, TX-4	Pending
12-069-101n	4/8/2012	GA-9, TX-2	Pending
12-075-102n	4/14/2012	AL-1, AR-2, MS-1, NC-1, SC-1, TX-4	Pending
12-074-107n	4/13/2012	TX-1	Pending
12-081-101n	4/20/2012	AL-1, TX-2	Pending

Appendix B: Materials, Methods, and Results for Molecular Analyses of MON 88701

B.1. Materials

The genomic DNA used in molecular analyses was isolated from leaf tissue of the R_3 generation of MON 88701 and the conventional control (Coker 130). The leaf tissue was harvested from a greenhouse production in 2010. For generational stability analysis, genomic DNA was extracted from leaf tissue of the R_2, R_3, R_4, R_5 and R_6 generations of MON 88701. The leaf tissue was harvested from production plan PPN-10-113. The reference substance, PV-GHHT6997 (Figure III-1), was used as a positive hybridization control in Southern blot analyses. Probe templates generated from PV-GHHT6997 were used as additional positive hybridization controls. As additional reference standards, the 1 Kb DNA Extension Ladder and λ DNA/*Hind* III Fragments from Invitrogen (Carlsbad, CA) were used for size estimations on agarose gels and subsequent Southern blots. The 1 Kb DNA Ladder from Invitrogen was used for size estimations on agarose gels for PCR analyses.

B.2. Characterization of the Materials

The identity of the source materials was verified by methods used in molecular characterization to confirm the presence or absence of MON 88701. The stability of the genomic DNA was confirmed by observation of interpretable signals from digested DNA samples on ethidium bromide stained agarose gels and/or specific PCR products, and the samples did not appear visibly degraded on the ethidium bromide stained gels.

B.3. DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA was isolated from MON 88701 leaf tissue using a hexadecyltrimethylammonium bromide (CTAB) based method. Briefly, 20 ml of CTAB buffer (1.5% w/v CTAB, 75 mM Tris HCl, 100 mM EDTA, 1.05 M NaCl, and 0.75% w/v PVP) and 10 mg RNase A were added to approximately 4 ml of ground leaf tissue and incubated at 60-70 °C for 40-50 min with intermittent mixing. Twenty milliliters of chloroform was added to the samples and mixed by hand for 2-3 min, then centrifuged at 10,300 × g for 8-10 min. The upper aqueous phase was put into a clean tube and the chloroform step was repeated twice. After the last chloroform step, the aqueous phase was put into a clean tube and the DNA was precipitated with 20 ml of 100% v/v ethanol. The sample was centrifuged for one minute to condense the pellet, and then the precipitated DNA was hooked out and put into a tube with 4-6 ml of 70% v/v ethanol to wash the DNA pellet. The samples were centrifuged at 5,100 × g for 5 min to pellet the DNA. DNA pellets were air dried, then resuspended in 250 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). All extracted DNA was stored in a -20 C freezer.

B.4. Quantification of DNA

Genomic DNA was quantified using a Qubit™ Fluorometer (Invitrogen, Carlsbad, CA).

B.5. Restriction Enzyme Digestion of DNA

Approximately 10 µg of genomic DNA extracted from MON 88701 and conventional control were digested with restriction enzyme *Bcl* I (New England Biolabs, Inc., Beverly, MA) or with restriction enzyme *Ssp* I- HF (New England Biolabs, Inc.). All *Bcl* I digests were conducted in 10X NEBuffer 3 buffer at 50 °C in a total volume of ~500 µl with ~50 units of restriction enzyme. All *Ssp* I-HF digests were conducted in 10X NEBuffer 4 at 37 °C in a total volume of ~500 µl with ~100 units of restriction enzyme. For the purpose of running positive hybridization controls, ~10 µg of genomic DNA extracted from the conventional control was digested with the restriction enzyme *Bcl* I and the appropriate positive hybridization control(s) were added to these digests prior to loading the agarose gel.

B.6. Agarose Gel Electrophoresis

Digested DNA was resolved on ~0.8% (w/v) agarose gels. For T-DNA insert/copy number and plasmid vector backbone analyses, individual digests containing ~10 µg each of MON 88701 and conventional control genomic DNA were loaded on the same gel in a long run/short run format. The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows for retaining the small molecular weight DNA on the gel. The positive hybridization controls were only run in the short run format. For the insert stability analysis, individual digests of ~10 µg each of genomic DNA extracted from five leaf samples from multiple generations of MON 88701 and the conventional control along with the positive hybridization controls were loaded on the agarose gel in a single run format.

B.7. DNA Probe Preparation for Southern Blot Analyses

Probe templates were prepared by PCR amplification using the PV-GHHT6997 DNA as template. The PCR products were separated on an agarose gel by electrophoresis and purified from the gel using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to manufacturer's instruction. The probe templates were designed based on the nucleotide composition (%GC) of the sequence in order to optimize the detection of DNA sequences during hybridization. When possible, probes possessing similar melting temperature (Tm) were combined in the same Southern blot hybridization. Approximately 25 ng of each probe template were radiolabeled with either [α-^{32}P] deoxycytidine triphosphate (dCTP) (6000 Ci/mmol) or [α-^{32}P] deoxyadenosine triphosphate (dATP) (6000 Ci/mmol) using RadPrime DNA Labeling System (Invitrogen, Carlsbad, CA) according to manufacturer's instruction.

B.8. Southern Blot Analyses of DNA

Genomic DNA isolated from MON 88701 and the conventional control was digested and evaluated using Southern blot analyses (Southern, 1975). The PV-GHHT6997 DNA, previously digested with the restriction enzyme *Pci* I was added to conventional control genomic DNA digested with *Bcl* I to serve as positive hybridization control on each Southern blot. When multiple probes were hybridized simultaneously to one Southern

blot, the probe templates were spiked in the digested conventional control genomic DNA to serve as additional positive hybridization controls on the Southern blot. The DNA was then separated by agarose gel electrophoresis and transferred onto a nylon membrane. Southern blots were hybridized and washed at 55 °C, 60 °C, or 65 °C, depending on the calculated melting temperature (Tm) of the probes that were used. Table B-1 lists the radiolabeling conditions and hybridization temperatures of the probes used in this study. Multiple exposures of each blot were then generated using Kodak Biomax MS film (Eastman Kodak, Rochester, NY) in conjunction with one Kodak Biomax MS intensifying screen in a -80 °C freezer.

Table B-1. Hybridization Conditions of Utilized Probes

Probe	Labeling Method	Element Sequence Spanned by DNA Probe	Probe labeled with dNTP (32P)	Hybridization/ Wash Temperature (°C)
1	RadPrime	B-Right Border, P-*PC1SV*, L-*TEV,* TS-*CTP2,* CS-*dmo* (portion)	dATP	55
2	RadPrime	TS-*CTP2* (portion), CS-*dmo* (portion)	dCTP	65
3	RadPrime	CS-*dmo* (portion), T-*E6*, P-*e35S*	dATP	55
4	RadPrime	P-*e35S* (portion), L-*Hsp70*, CS-*bar*, T-*nos* (portion)	dCTP	65
5	RadPrime	CS-*bar* (portion) , T-nos, B-Left Border	dATP	55
6	RadPrime	OR-*ori V*	dCTP	60
7	RadPrime	CS-*rop,* OR-*ori-pBR322* (portion)	dCTP	60
8	RadPrime	OR-*ori-pBR322* (portion)*, aadA*	dCTP	60

B.9. DNA Sequence Analyses of the Insert

Overlapping PCR products, denoted as Product A, Product B, and Product C, were generated that span the insert and adjacent 5′ and 3′ flanking DNA sequences in MON 88701. These products were analyzed to determine the nucleotide sequence of the insert in MON 88701, as well as that of the DNA flanking the 5′ and 3′ ends of the insert.

The PCR analysis for Product A was conducted using ~100 ng of genomic DNA template in a 50 µl reaction volume. The reaction volume contained either a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM of each dNTP, and 0.02 units/µl of Accuprime *Taq* DNA Polymerase High Fidelity (Invitrogen) or a final concentration of, 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP, and 0.02 units/µl of Phusion Hot Start II High Fidelity DNA Polymerase (Finnzymes, Espoo, Finland). The Phusion Hot Start II High Fidelity DNA Polymerase was used to enhance the amplification and sequencing of a small A-rich region located in Product A.

The PCR analyses for Product B and Product C were each conducted using ~100 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM of each dNTP, 2.5% (v/v) DMSO, and 0.02 units/µl of Accuprime *Taq* DNA Polymerase High Fidelity (Invitrogen).

The amplification of Product A using Accuprime *Taq* was performed under the following cycling conditions: 1 cycle at 94 °C for 2 minutes; 35 cycles at 94 °C for 15 seconds, 59 °C for 30 seconds, 68 °C for 2.25 minutes; 1 cycle at 68 °C for 5 minutes. The amplification of Product A using Phusion Hot Start II DNA Polymerase was performed under the following cycling conditions: 1 cycle at 98 °C for 30 seconds, 35 cycles at 98 °C for 10 seconds, 64 °C for 15 seconds, and 72 °C for 1.25 minutes. The amplification of Product B was performed under the following cycling conditions: 1 cycle at 94 °C for 2 minutes; 35 cycles at 94 °C for 15 seconds, 50 °C for 30 seconds, 68 °C for 3 minutes; 1 cycle at 68 °C for 5 minutes. The amplification of Product C was performed under the following cycling conditions: 1 cycle at 94 °C for 2 minutes; 35 cycles at 94 °C for 15 seconds, 62 °C for 30 seconds, 68 °C for 2 minutes; 1 cycle at 68 °C for 5 minutes.

A small aliquot of each PCR product was separated on a 1.0% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the products were the expected size. Prior to sequencing, each verified PCR product was purified using the QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA) and quantified using a Qubit fluorometer. The purified PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by Monsanto TGAC (The Genome Analysis Center) using BigDye terminator chemistry (Applied Biosystems, Foster City, CA).

A consensus sequence was generated by compiling multiple sequencing reactions performed on the overlapping PCR products. This consensus sequence was aligned to the

PV-GHHT6997 sequence to determine the integrity and organization of the integrated DNA and the 5′ and 3′ insert-to-flank DNA junctions in MON 88701.

B.10. PCR and DNA Sequence Analysis to Examine the MON 88701 Insertion Site

To examine the MON 88701 insertion site in conventional cotton, PCR and sequence analyses were performed on genomic DNA from both MON 88701 and conventional cotton. The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 88701. A forward primer specific to the DNA sequence flanking the 5′ end of the insert was paired with a reverse primer specific to the DNA sequence flanking the 3′ end of the insert.

The PCR reactions were conducted using ~100 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM of each dNTP, and 0.02 units/µl of Accuprime *Taq* DNA Polymerase High Fidelity (Invitrogen). The amplification was performed under the following cycling conditions: 1 cycle at 94 °C for 2 minutes; 35 cycles at 94 °C for 15 seconds, 58 °C for 30 seconds, 68 °C for 30 seconds; 1 cycle at 68 °C for 5 minutes.

A small aliquot of each PCR product was separated on a 1.2% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the PCR products were the expected size prior to sequencing. Only the verified PCR product from the parental conventional control was purified with the QIAquick PCR Purification Kit (Qiagen) and quantified using a Qubit Fluorometer. The purified PCR product was sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto TGAC (The Genome Analysis Center) using BigDye terminator chemistry (Applied Biosystems).

A consensus sequence was generated by compiling multiple sequencing reactions performed on the verified PCR product. This consensus sequence was aligned to the 5′ and 3′ sequences flanking the MON 88701 insert to determine the integrity and organization of the insertion site.

References for Appendix B

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98:503-517.

Appendix C: Protein Reaction Products, Materials, Methods, and Results for Characterization of MON 88701 DMO and PAT (*bar*) Proteins Produced in MON 88701, and Substrate Specificity

C.1. DMO Reaction Products

MON 88701 when treated with dicamba herbicides will yield the reaction products 3,6-dichlorosalicylic acid (DCSA) and formaldehyde during demethylation of the herbicide. These products, as you will see in the text below, have either been previously deemed safe (DCSA) or are commonly produced in nature and at sufficiently low levels in this MON 88701 cropping system (formaldehyde) so as to not raise concerns with regard to the plant pest risk assessment for MON 88701.

C.1.1. DCSA in MON 88701

DCSA is a metabolite generated when dicamba herbicide is sprayed on MON 88701 cotton and soybean and is also produced by livestock and soil whose safety has been evaluated by the Environmental Protection Agency (U.S. EPA, 2009; FAO-WHO, 2011). DCSA residue levels were measured in dicamba-treated MON 88701 to support Monsanto's registration request for the inclusion of DCSA in the cottonseed and gin by-product dicamba residue definitions. DCSA is structurally similar to salicylic acid (SA). Numerous studies have reported on the stress defense activities of SA, although most studies have looked at the protective effects of exogenously applied SA (Janda et al, 2007).

C.1.2. Formaldehyde in the Environment

Formaldehyde is ubiquitous in the environment; plants and animals are constantly exposed to low levels already present in the environment and the atmosphere from a variety of biogenic (*e.g.*, plant and animal) and anthropogenic (*e.g.*, automotive or industrial emissions) sources. In water, formaldehyde dissipates through biodegradation to low levels in a few days (USHHS-ATSDR, 1999). Aerobic biodegradation half-lives are estimated to be 1-7 days for surface water and 2-14 days for ground water (U.S. EPA, 2008). The half-life of formaldehyde in air is dependent on a number of factors (light intensity, temperature, and location). Through reaction with hydroxyl radical, the half-life of formaldehyde in air varies from 7 to 70 hours (U.S. EPA, 2008). The photolytic half-life of formaldehyde in air (*e.g.*, in the presence of sunlight) is estimated to be 1.6-6 hours (U.S. EPA, 2008; USHHS-ATSDR, 1999). Formaldehyde is rapidly consumed in the atmosphere through direct photolysis or by oxidation with hydroxyl or nitrate radicals (USHHS-ATSDR, 1999).

Humans are constantly exposed to low levels of formaldehyde. Human exposure to formaldehyde is primarily due to indoor air exposures (USHHS-ATSDR, 1999). Formaldehyde is found in a variety of consumer products such as cosmetics and paints, often as an antimicrobial agent, and is used extensively in urea-formaldehyde "slow-release" fertilizer formulations and adhesives (USHHS-ATSDR, 1999). Indoor formaldehyde air concentrations are generally significantly higher than outdoor air

concentrations (USHHS-ATSDR, 1999) as a result of combustion (cooking, heating, tobacco use) and the emission of formaldehyde from a variety of construction materials (*e.g.*, particle board, plywood or foam insulation) as well as permanent press fabrics (*e.g.*, clothing or draperies) (U.S. CPSC, 1997). Formaldehyde present in outdoor air results from a number of sources, and levels of formaldehyde are generally higher in urban areas than in rural areas (WHO-IPCS, 1989). Direct contributions of formaldehyde to the atmosphere (*i.e.*, those in the form of formaldehyde itself) from man-made sources are present, but are generally considered to be small relative to natural sources or indirect production of formaldehyde in the atmosphere (WHO, 2002).

C.1.3. Formaldehyde in MON 88701

Formaldehyde is a metabolite when dicamba is sprayed on MON 88701 cotton. However, formaldehyde is not considered a relevant metabolite in the demethylation of dicamba by U.S. EPA. According to the guidelines published by Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency (US EPA OPPTS 860.1300), the methoxy side chain that is cleaved from dicamba to form formaldehyde would specifically not be chosen to be labeled in a metabolism study (U.S. EPA, 1996). This is because it is not metabolically stable and would not be considered a significant moiety as it would be readily metabolized and incorporated into the 1-carbon pool of the plant through known pathways. Therefore, formaldehyde was not measured in the residue study when dicamba was applied to MON 88701.

The maximum theoretical production of formaldehyde produced from dicamba-treated MON 88701 is estimated to be 6.3 mg/kg and 33 mg/kg[7]. This is well within the range of formaldehyde concentrations measured for a variety of agricultural commodities, including up to 60 mg/kg in fruits and vegetables (WHO-IPCS, 1989). Plants have a large capacity to metabolize formaldehyde naturally produced from internal processes (A. Hanson (2011), [redacted] Eminent Scholar, Horticulture Department, University of Florida, Personal Communication), and any additional amount of formaldehyde that could be theoretically produced in the plant by dicamba treatment in MON 88701 would be metabolized very quickly. Thus the incremental increase in formaldehyde over and above the levels already presumed to be present in the cotton plant would be small and transient and associated with an outdoor application of dicamba herbicide. Further, since current literature supports that formaldehyde is only emitted from foliage under certain

[7] Calculation based an assumption that the entire 0.56 kg/ha (0.5 lb/acre a.e.) application of dicamba that is intercepted by the MON 88701 cotton plant at the 6-leaf or first bloom plus 15 day growth stage is instantaneously and completely absorbed, and then instantaneously metabolized by the DMO enzyme (Complete demethylation of 560 g (2.5 mol)/ ha dicamba would yield 2.5 mol/ha formaldehyde). Canopy closure, and thus spray interception, is estimated at 30% at the 6-leaf stage (Krutz et al., 2012), resulting in production of 23 g/ha formaldehyde. Canopy closure is near complete at the first bloom plus 15 day growth stage (Reddy et al., 2009), so no adjustment is applied. Above-ground biomass of 6-leaf plants is estimated to be 0.7 metric tons/ha (Ducamp et al., 2012), and the estimated maximum theoretical concentration is 33 mg/kg formaldehyde *in planta*. For dicamba applications at first bloom plus 15 day growth stage, the crop biomass is estimated to be 12 metric tons/ha (Boquet and Breitenbeck, 2000), and the estimated maximum theoretical formaldehyde concentration produced *in planta* is 6.3 mg/kg.

conditions (Nemecek-Marshall et al., 1995; Cojocariu et al., 2004; Cojocariu et al., 2005) and that emission rates are low (Nemecek-Marshall et al., 1995), little opportunity exists for formaldehyde to be released from MON 88701 after dicamba treatment. Therefore human safety concerns of formaldehyde released from dicamba-treated MON 88701 are considered to be negligible and the most relevant route of exposure is from repeated inhalation of concentrated levels associated with indoor or occupational environments. USHHS-NTP (2011) has already stated that there is no evidence to suggest that dietary intake of formaldehyde is important, despite NTP's 12th Report on Carcinogens reclassifying formaldehyde as a known human carcinogen by (USHHS-NTP, 2011). In addition, the only human food currently produced from cottonseed is refined, bleached, and deodorized (RBD) oil, and to a smaller extent, linters. Therefore, the potential for human exposure to any formaldehyde in dicamba-treated MON 88701 cottonseed is highly unlikely.

C.1.4. Conclusion

Data from both dicamba and glufosinate-treated and not treated MON 88701 compared to a conventional control are available from multiple sites across the U.S., where agronomic, phenotypic and environmental interaction data were collected. The results of this assessment demonstrate no biologically meaningful difference between MON 88701 treated with and without dicamba and glufosinate and the conventional control, and support a conclusion that the formation of DCSA and formaldehyde does not alter the weedy characteristics or increase susceptibility or tolerance to diseases, insect pests or abiotic stresses. Therefore, MON 88701, as cultivated, is no more likely to be a plant pest risk or have a biologically meaningful change in environmental impact than conventional cotton.

C.2. Characterization of MON 88701 DMO Protein in MON 88701

C.2.1 Forms of DMO

Various forms of the DMO protein (Figure C-1) were used to establish enzyme structure, activity, substrate specificity and safety of the proteins in MON 88701. The wild-type DMO was first isolated and characterized from *Stenotrophomonas maltophilia* (Herman et al., 2005). The MON 88701 DMO protein present in MON 88701 is identical to the wild-type DMO, except for an additional leucine at position two and an additional nine amino acids at the N-terminus from the chloroplast transit peptide, CTP2 (Figure C-1). The *E. coli*-produced form of DMO is identical to the wild-type DMO, but with a histidine-tag on the N-terminus (Figure C-1), was used for specificity experiments. The differences in the amino acid sequence or the addition of N-terminal histidine tag did not appear to have an effect on mode-of-action, structure, functional activity, or specificity of DMO, as these changes are sterically distant from the catalytic domain centers involved in electron transport (Rieske and non-heme iron centers) and the catalytic centers for the dicamba substrate (D'Ordine et al., 2009; Dumitru et al., 2009).

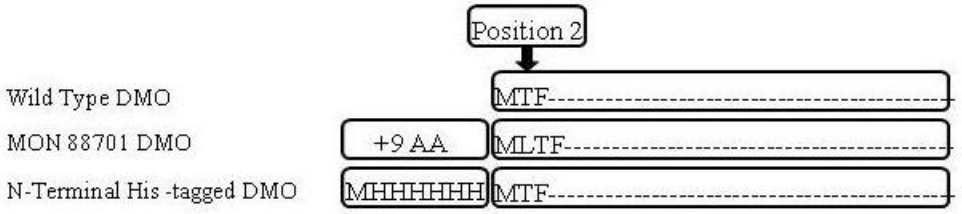

Figure C-1. Forms of DMO Protein and Their Relation to the Wild-Type DMO Protein

The diagram represents the various DMO forms described in this petition. The wild-type DMO form isolated from *S. maltophilia* was the first form sequenced (Herman et al., 2005). The MON 88701 DMO protein has an insertion of a leucine at position 2, and the addition of 9 amino acids from CTP2 at the N-terminus. MON 88701 DMO was purified from cottonseed of MON 88701. *E. coli*-produced MON 88701 DMO has the same sequence as plant-produced MON 88701 DMO; equivalence between the two proteins has been demonstrated (Section V.B and Appendix C). The N-terminal histidine-tagged DMO was produced in *E. coli* and was used for *in vitro* specificity studies (Section V.A.1.2).

C.2.2. Materials

The MON 88701 DMO protein (lot 11299151) was purified from cottonseed of MON 88701 (lot 11287350). The MON 88701 DMO protein was stored in a -80 ºC freezer in a buffer solution containing 50 mM potassium phosphate, pH 8.0, 1 mM DTT, 1 mM benzamidine HCl, 0.1 M sodium chloride and 10% glycerol.

The *E. coli*-produced MON 88701 DMO protein (lot 11300031) was used as the reference substance. The DMO protein reference substance was generated from cell paste produced by large-scale fermentation of *E. coli* containing the pMON136400 expression plasmid. The coding sequence for *dmo* contained on the expression plasmid (pMON136400) was confirmed prior to and after fermentation. The *E. coli*-produced MON 88701 DMO protein was previously characterized.

C.2.3. Description of Assay Control

Protein MW standards (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were used to calibrate some SDS-PAGE gels and verify protein transfer to polyvinylidene difluoride (PVDF) and nitrocellulose membranes. Broad Range SDS-PAGE MW standards (Bio-Rad, Hercules, CA) were used to generate a standard curve for the apparent MW estimation. Bovine serum albumin (BSA) and α-aminobutyric acid (AAbA) were used as hydrolysis control and internal calibration standard for amino acid analysis. The *E. coli*-produced MON 88701 DMO reference standard was used to construct a standard curve for the estimation of total protein concentration using a Bio-Rad protein assay. A phenylthiohydantoin (PTH) amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to calibrate the

Applied Biosystems 494 Procise Sequencing System for each analysis. A peptide mixture (Sequazyme Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. Transferrin (Sigma-Aldrich, St. Louis, MO) was used as positive control for glycosylation analysis.

C.2.4. Protein Purification

The MON 88701 DMO was purified from cottonseed of MON 88701. The purification procedure was not performed under a GLP plan; however, all procedures were documented on worksheets and, where applicable, SOPs were followed. The MON 88701 DMO protein was purified from an extract of ground cottonseed using a combination of ammonium sulfate precipitation, hydrophobic interaction chromatography, anion exchange chromatography, mixed mode ion exchange chromatography and size exclusion chromatography. The purification procedure is briefly described below.

Approximately 1 kg of MON 88701 cottonseed expressing the DMO protein was mixed with ~1 kg of dry ice and ground to fine powder using a laboratory mill (model 3100, Perten Instruments). The ground powder was suspended in two liters of hexane (EMD Chemicals Inc., Gibbstown, NJ) and filtered. This process was repeated four times in order to completely defat the powder. After drying overnight, the powder was ready for further processing. All grinding and defatting steps were done in a fume hood at room temperature.

The ground powder was mixed with extraction buffer (50 mM Tris, pH 8.0, 2.0 M deionized urea, 0.2 M boric acid, 1.0 mM dithiothreitol (DTT), 1.0 mM benzamidine-HCl, 1.0 µm bestatin, 1.0 µM E-64 and Complete EDTA free protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN)) to a final volume of 8 liters and incubated for 2 h at room temperature. The slurry was centrifuged at 15000 × g for 30 min at 4 °C. The supernatant was collected and brought to 0.05% polyethyleneimine (PEI). The solution was stirred at ~4 °C for 30 min and then centrifuged at 15000 × g for 30 min. The supernatant was collected and ~2.2 kg of ammonium sulfate was slowly added to bring the solution to 50% w/v ammonium sulfate saturation. This solution was stirred at ~4 °C for 2 h and the pellet was collected by centrifugation at 15000 × g for 30 min. The pellet was resuspended in 10 liters of the resuspension buffer (50 mM Tris-HCl, pH 8.0, 0.35 M ammonium sulfate, 10 mM DTT, 1.0 mM benzamidine-HCl, 1.0 µM bestatin, 1.0 µM E-64 and Complete EDTA free protease inhibitor cocktail). The solution was stirred in the cold room overnight and then centrifuged at 30,000 × g for 1 h. Supernatant was collected and loaded onto a 1 liter butyl sepharose column (GE Healthcare) equilibrated with butyl sepharose equilibration (BSE) buffer (50 mM Tris-HCl, pH 8.0, 0.35 M ammonium sulfate, 1 mM DTT, 1.0 mM benzamidine-HCl, 1.0 µM bestatin, 1.0 µM E-64 and Complete EDTA-free protease inhibitor cocktail). All column steps were run at room temperature. The column was washed with 5 liters BSE buffer. Proteins were eluted with 1 liter of buffer containing 25 mM Triethanolamine, pH 8.0, 100 µM dicamba, 1.0 mM DTT, 1.0 mM benzamidine-HCl, 1.0 µM bestatin, 1.0 µM E-64 and Complete EDTA free protease inhibitor cocktail (BSEL buffer). After

eluting the proteins with 1 liter of BSEL buffer, the flow was stopped for one hour and then elution was continued with additional 1 liter of BSEL buffer. Both elutions were pooled and loaded onto a 25 ml DEAE macroprep column (Bio Rad) equilibrated with DEAE macroprep equilibration (DME) buffer (50 mM Tris-HCl, pH 8.0, 100 µM dicamba, 1.0 mM DTT, 1.0 mM benzamidine-HCl, 1.0 µM bestatin, 1.0 µM E-64 and Complete EDTA free protease inhibitor cocktail). All steps associated with DEAE macroprep were performed at ~4 °C. The column was washed with 125 ml DME buffer and proteins were eluted with 75 ml DME buffer containing 70 mM NaCl and then with a linear gradient that increased from 70 mM to 350 mM NaCl over 500 ml. Fractions containing MON 88701 DMO were pooled and loaded onto a 2.5 ml ceramic hydroxyapatite (CHT) column (Bio-Rad) equilibrated with CHT equilibration buffer (50 mM Tris-HCl, pH 8.0, 100 µM dicamba, 1.0 mM DTT, 1.0 mM benzamidine-HCl, 1.0 µM bestatin, 1.0 µM E-64 and Complete EDTA free protease inhibitor cocktail). All steps associated with CHT were performed at ~4 °C. Most of the MON 88701 DMO was found in the flow through and wash fractions. Flow through and wash fractions from CHT were pooled separately (Pooled FT and Pooled Wash, respectively) and reloaded on two separate CHT columns as follows.

The Pooled FT was loaded onto a ~10 ml CHT column (CHT3). The column was washed with 50 ml of CHT equilibration buffer and step eluted using the CHT equilibration buffer containing 1 mM, 2 mM and 3 mM potassium phosphate, pH 8.0. The Pooled Wash was loaded onto a ~3 ml CHT column (CHT2). CHT2 was washed with ~45 ml of CHT equilibration buffer and step eluted using the CHT equilibration buffer containing 1 mM, 2 mM and 3 mM potassium phosphate, pH 8.0.

Wash fractions from both the CHT2 and CHT3 chromatography runs that contained MON 88701 DMO were pooled and loaded onto a ~1 ml DEAE macroprep column equilibrated with DME buffer for concentration. The column was washed with 10 ml of the DME buffer and eluted with DME buffer containing 500 mM NaCl. The MON 88701 DMO containing fractions were pooled and loaded onto a Hi-Prep Sephacryl S 100 size exclusion column equilibrated at ~4 °C with 50 mM potassium phosphate, pH 8.0, 1 mM DTT, 1 mM benzamidine-HCl, 0.1 M NaCl and 10% (v/v) glycerol. Fractions containing MON 88701 DMO were pooled and concentrated with aquacide (EMD Biosciences, Inc., La Jolla, CA) at ~4 °C to a final volume of 750 µl.

Elution fractions (1-3 mM potassium phosphate, pH 8.0 fractions) from both the CHT2 and CHT3 that contained MON 88701 DMO were pooled and concentrated using a Amicon ultra spin concentrator (Millipore, Bedford, MA) with a 10K MWCO. The centriprep concentrated pool was then loaded onto a Hi Prep Sephacryl S 100 size exclusion column equilibrated at ~4 °C with 50 mM potassium phosphate, pH 8.0, 1 mM DTT, 1 mM benzamidine-HCl, 0.1 mM NaCl and 10% (v/v) glycerol. Fractions containing MON 88701 DMO were pooled and concentrated with aquacide at ~4 °C to a final volume of 750 µl.

Both aquacide concentrated samples were pooled to a final volume of 1.5 ml. The final buffer composition of the purified MON 88701 DMO protein was 50 mM potassium phosphate, pH 8.0, 1 mM DTT, 1 mM benzamidine-HCl, 100 mM sodium chloride and

10% (v/v) glycerol. This MON 88701 DMO purified from the cottonseed of MON 88701 was aliquoted and stored in a -80 °C freezer.

C.2.5. Summary of DMO Protein Identity and Equivalence

Table C-1. Summary of MON 88701 DMO Protein Identity and Equivalence

Analytical Test Assessment	Analytical Test Outcome
1. N-terminal sequence analysis of the MON 88701 DMO protein to assess identity	• The identity could not be confirmed by N-terminal sequence analysis • MALDI-TOF MS[1] analysis of peptides derived from tryptic digested MON 88701 DMO established the N-terminal sequence of MON 88701 DMO
2. MALDI-TOF MS[1] analysis of peptides derived from tryptic digested MON 88701 DMO protein to assess identity	• MALDI-TOF MS[1] analysis yielded peptide masses consistent with the expected peptide masses from the theoretical trypsin digest of the MON 88701 DMO sequence
3. Western blot analysis using anti-DMO polyclonal antibodies to assess identity and immunoreactive equivalence between MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins	• MON 88701 DMO protein identity was confirmed using a western blot probed with antibodies specific for DMO protein • Immunoreactive properties of the MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins were shown to be equivalent
4. SDS-PAGE[2] to assess equivalence of the apparent molecular weight between MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins	• Electrophoretic mobility and apparent molecular weight of the MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins were shown to be equivalent
5. Glycosylation analysis of the MON 88701 DMO protein to assess equivalence between the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins	• Glycosylation status of MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were shown to be equivalent
6. DMO enzymatic activity analysis to assess functional equivalence between MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins	• Functional activity of the MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins were shown to be equivalent

[1]MALDI-TOF MS = Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
[2]SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis

C.2.6. N-Terminal Sequencing

C.2.6.1. Methods

N-terminal sequencing by automated Edman degradation chemistry was carried out in an attempt to confirm the identity of MON 88701 DMO.

MON 88701 DMO was separated by SDS-PAGE and transferred to PVDF membrane. The blot was stained using Coomassie Blue R-250. The major band at ~39 kDa containing MON 88701 DMO was excised from the blot and was used for N-terminal sequence analysis. The analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapiller et al., 1983) using an Applied Biosystems 494 Procise Sequencing System equipped with 140C Microgradient system a Perkin Elmer Series 200 UV/VIS Absorbance Detector with Procise™ Control Software (version 2.1) for amino acid detection after each cycle. Chromatographic data were collected using SequencePro (version 2.1) software. A control protein, β-lactoglobulin, (Applied Biosystems, Foster City, CA) was analyzed before and after the sequence analysis of the MON 88701 DMO protein to verify that the sequencer met performance criteria for repetitive yield and sequence identity. Identity was established if ≥ 8 amino acids, consistent with the predicted sequence of the N-terminus of the MON 88701 DMO, were observed during analysis.

C.2.6.2. Results of the N-terminal Sequence Analysis

N-terminal sequencing reaction was performed on MON 88701 DMO protein. The reaction did not yield any observable sequence presumably because the N-terminus was blocked. Although this analysis did not yield N-terminal sequence data, the N-terminus of the MON 88701 DMO protein was determined using MALDI-TOF tryptic mass map analysis (see Section C.2.6).

C.2.7. MALDI-TOF Tryptic Mass Map Analysis

C.2.7.1. Methods

MALDI-TOF tryptic mass fingerprint analysis was used to confirm the identity of the MON 88701 DMO protein. MON 88701 DMO protein (~15 µg) was chilled in a -20 °C freezer for at least 10 min. The chilled protein was precipitated with 200 µl of 95% acetone in a -20 °C freezer overnight. Precipitated protein sample was pelleted in a refrigerated centrifuge for at least 45 min at more than 13,000 × g. The supernatant was carefully removed and discarded. The protein pellet was washed twice with 200 µl of chilled ethanol to remove residual supernatant. The pellet was dried to completion using a Speed Vac concentrator and resuspended in 30 µl of 40% 2,2,2,-trifluoroethanol (TFE) in 25 mM ammonium bicarbonate. The resuspended protein was vortexed vigorously and then sonicated for 5 min in a water bath. The sample was incubated at ~37 °C for 1 h to denature the proteins. Denatured protein sample was reduced with ~5 mM tris(2-carboxyethyl)phosphine (TCEP) for 1 h at ~37 °C. Reduced protein sample was then alkylated in the dark for 30 min at room temperature with ~10 mM iodoacetic acid. Additional TCEP was added to ~5 mM and the sample was incubated for 10 min at room temperature. The reduced and denatured test substance was mixed with 67 µl of 25 mM ammonium bicarbonate and 2.5 µl of trypsin solution (0.2 µg/µl in 25 mM ammonium bicarbonate). The tryptic digestion was allowed to proceed for 15 h at 37 °C followed by quenching with 1 µl of formic acid. Proteolytic peptides were dried to completion using Speed Vac concentrator. To solubilize the dried peptides, a solution of 50% acetonitrile, 0.1% TFA was added and sonicated for 5 min. Aliquots from the digest were spotted to

three wells on an analysis plate. For each spot, 0.75 µl of 2, 5 dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (α-Cyano), or 3, 5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) (Thermo Fisher Scientific Inc.) was added to one of the spots. The sample in DHB matrix was analyzed in the 300 to 5000 Da range. Samples in α-Cyano and Sinapinic acid were analyzed in the 500 to 5000 Da and 500 to 7000 Da range, respectively. The analysis was performed using a Voyager™ DE Pro Biospectrometry™ workstation (Applied Biosystems) using Voyager Instrument Control Panel software (version 5.10.2) and Data Explorer data analysis software (version 4.0.0.0). Protonated peptide masses were monoisotopically resolved 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 (Lighthouse Data, Odense M, Denmark) was used to generate a theoretical trypsin digest of the MON 88701 DMO protein sequence. Masses within 1 Da of a monosiotopic mass were matched against the theoretical digest of the MON 88701 DMO sequence. All matching masses were tallied and a coverage map was generated for the mass fingerprint. The tryptic mass fingerprint coverage was considered acceptable if ≥ 40% of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments (Biron et al., 2006, Krause et al., 1999).

C.2.7.2. Results of MALDI-TOF Tryptic Mass Map Analysis

The identity of the MON 88701 DMO protein was confirmed by MALDI-TOF MS analysis of peptide fragments produced from tryptic digestion of the MON 88701 DMO protein. The ability to identify a protein using this method is dependent upon matching a sufficient number of observed tryptic peptide fragment masses with predicted tryptic peptide fragment masses. In general, protein identification made by peptide mapping is considered to be reliable if ≥ 40% of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments (Biron et al., 2006, Krause et al., 1999).

There were 19 unique peptides identified that corresponded to the masses expected to be produced by tryptic digestion of the MON 88701 DMO protein (Table C-2). The identified masses were used to assemble a coverage map of the entire MON 88701 DMO protein (Figure C-2). The experimentally determined mass coverage of the MON 88701 DMO protein was 66.5% (232 out of 349 amino acids). This analysis serves as identity confirmation for the MON 88701 DMO protein.

To identify the N-terminus, the experimentally determined masses of the peptides produced from tryptic digestion of the MON 88701 DMO protein were examined for the presence of a mass that matched the theoretical mass expected from the MON 88701 DMO protein deduced from the *dmo* gene present in MON 88701. A mass was identified that corresponded to the predicted mass of an acetylated peptide with nine amino acids from CTP2 followed by the MON 88701 DMO protein deduced from the *dmo* gene present in MON 88701. The additional nine amino acids of CTP2 resulted from the alternative processing of CTP2. Alternative processing of DMO precursor proteins has been observed in other dicamba-tolerant plants containing the *dmo* gene (Behrens et al.,

2007). Hence, the MON 88701 DMO protein was designated to have an N-terminal end as shown in Figure C-2.

Table C-2. Summary of the Tryptic Masses[1] Identified for the MON 88701 DMO Protein Using MALDI-TOF MS

α-cyano	DHB	Sinapinic acid	Expected Mass	Diff.[2]	Fragment[3]	Sequence
720.40			720.37	0.03	140-145	VDPAYR
833.51	833.45		833.45	0.06	108-114	SFPVVER
856.49			856.43	0.06	251-257	EQSIHSR
914.60			914.53	0.07	305-312	VVVEAIER
	1030.58		1030.57	0.01	293-301	SWQAQALVK
1108.61	1108.59		1108.50	0.11	176-185	ANAQTDAFDR
1275.87	1275.83		1275.73	0.14	35-45	TILDTPLALYR
1286.83			1286.70	0.13	302-312	EDKVVVEAIER
1428.84	1428.83		1428.69	0.15	218-230	GANTPVDAWNDIR
	1470.74		1470.63	0.11	146-158	TVGGYGHVDCNYK
	1501.91		1501.79	0.12	189-202	EVIVGDGEIQAALMK
	1506.86		1506.73	0.13	176-188	ANAQTDAFDRLER
	1577.89	1577.80	1577.73	0.16	279-292	NFGIDDPEMDGVLR
		1731.92	1731.80	0.12	1-15	VMSSVSTACMLTFVR +42 Da (N-acetylation)
	1745.09	1744.99	1744.93	0.16	234-250	VSAMLNFIAVAPEGTPK
	1994.30	1994.23	1994.03	0.27	159-175	LLVDNLMDLGHAQYVHR
		2143.35	2143.12	0.23	16-34	NAWYVAALPEELSEKPLGR
	2398.37	2398.35	2398.09	0.28	258-278	GTHILTPETEASCHYFFGSSR
		2724.72	2724.31	0.41	115-139	DALIWIWPGDPALADPGAIPGCR

[1]Only experimental masses that matched expected masses are listed in the table.
[2]The difference between the expected mass and the first column mass. Other masses shown within a row are also within 1 Da of the expected mass.
[3]Position refers to amino acid residues within the predicted MON 88701 DMO sequence as depicted in Figure C-2.
DHB = 5-dihydroxybenzoic acid matrix, α-cyano = α-cyano-4-hydroxycinnamic acid matrix, Sinapinic acid = 3, 5-dimethoxy-4-hydroxycinnamic acid matrix.

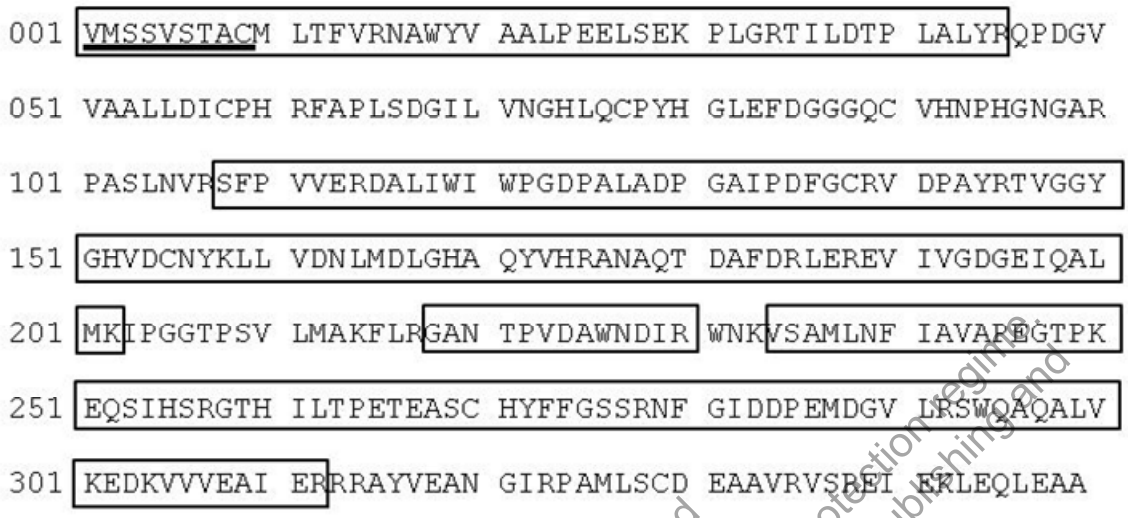

Figure C-2. MALDI-TOF MS Coverage Map of the MON 88701 DMO Protein
The amino acid sequence of the MON 88701 DMO protein was deduced from the *dmo* gene present in MON 88701. Boxed regions correspond to regions covered by tryptic peptides that were identified from the MON 88701 DMO protein sample using MALDI-TOF MS. Underlined region corresponds to the nine amino acids from CTP2 retained at the N-terminus of the MON 88701 DMO. In total, 66.5% (232 of 349 total amino acids) of the expected protein sequence was covered by the identified peptides.

C.2.8. Western Blot Analysis-Immunoreactivity

C.2.8.1. Methods

Western blot analysis was performed to confirm the identity of the MON 88701 DMO protein purified from cottonseed of MON 88701 and to compare the immunoreactivity of the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins.

The MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were analyzed concurrently on the same gel using three loadings of 0.5, 2, and 6 ng. Loadings of the three concentrations were made in duplicate on the gel. Aliquots of each protein were diluted in water and 5X Laemmli buffer (LB) containing 312 mM Tris-HCl, 25% (v/v) 2-mercaptoethanol, 10% (w/v) SDS, 0.025% (w/v) bromophenol blue, 50% (v/v) glycerol, pH 6.8, heated at 101 °C for 3 min, and applied to a 15 well pre-cast Tris-glycine 4-20% polyacrylamide gradient gel (Invitrogen, Carlsbad, CA). Pre-stained molecular weight markers (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were loaded in parallel to verify electrotransfer of the proteins to the membrane and to estimate the size of the immunoreactive bands observed. Electrophoresis was performed at a constant voltage of 150 V for 90 min. Electrotransfer to a 0.45 µm nitrocellulose membrane (Invitrogen, Carlsbad, CA) was performed for 105 min at a constant voltage of 25 V. After electrotransfer, the membrane was stored overnight with 1× phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBST) at 4 °C. The membrane was blocked for 1 h with 5% (w/v) NFDM in PBST at room temperature. The membrane was then probed with a 1:5000 dilution of goat anti-DMO antibody (lot 11223358) in 2% NFDM in PBST for 1 h at room temperature. Excess antibody was removed using two 1 min washes followed by three 5 min washes with PBST. Finally, the membrane was probed with horseradish peroxidase (HRP)-conjugated horse anti-goat IgG (Thermo, Rockford, IL) at a dilution of 1:10,000 in 2% NFDM in PBST for 1 h at room temperature. Excess HRP-conjugate was removed using two 1 min washes and three 5 min washes with PBST. All washes were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (GE, Healthcare, Piscataway, NJ) with exposure to Amersham Hyperfilm ECL (GE, Healthcare, Piscataway, NJ). The film was developed using a Konica SRX-101A automated film processor (Konica Minolta Medical & Graphic, Inc., Tokyo, Japan).

Quantification of the bands on 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 band location and volume tool. The signal intensities of the immunoreactive bands observed for the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins migrating at the expected position on the blot film were quantified as "adjusted volume" values. The raw data was exported to a Microsoft Excel (2007) file. The immunoreactivity of the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were reported as the mean signal intensity at each amount of protein analyzed. The immunoreactivity of the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were considered equivalent if the overall mean of the immunoreactive signal of the MON 88701 DMO protein was within ± 35% of the overall mean of immunoreactive signal of the *E. coli*-produced MON 88701 DMO protein.

C.2.8.2. Results of MON 88701 DMO Protein Immunoreactivity Equivalence

Western blot analysis was conducted using goat anti-DMO polyclonal antibodies to 1) assess the identity of the MON 88701 DMO protein isolated from the cottonseed of MON 88701 and 2) determine the relative immunoreactivity of the MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins. The results demonstrated that the anti-DMO antibodies recognized the MON 88701 DMO protein that migrated to the same position on the blot as the *E. coli*-produced MON 88701 DMO protein (Figure C-3). Furthermore, the immunoreactive signal increased with increasing amounts of MON 88701 DMO protein loaded. Two other bands, one migrating at ~75 kDa and the other at ~17 kDa were also observed. These bands were prominent in lanes with higher load amounts (Figure C-3, Lanes 3-6), and may represent products of aggregation and degradation of DMO, respectively.

Densitometric analysis was conducted to compare the immunoreactivity of MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins. The mean signal intensity ($OD \times mm^2$) from the MON 88701 DMO bands and from the *E. coli*-produced MON 88701 DMO bands at each amount of protein analyzed was calculated and then overall mean signal intensity was calculated (Table C-3). The immunoreactivity was considered equivalent if the overall mean signal intensity of all MON 88701 DMO protein bands was within ±35% of the overall mean signal intensity of *E. coli*-produced MON 88701 DMO protein bands across all loading levels.

The overall mean signal intensity of the *E. coli*-produced MON 88701 DMO bands was 6.500 $OD \times mm^2$, and the overall mean signal intensity of the MON 88701 DMO bands was 4.440 $OD \times mm^2$. Because overall mean signal intensity of the MON 88701 DMO protein bands was between 4.225 and 8.775 (between -35% and +35% of the *E. coli*-produced MON 88701 DMO bands), the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were determined to have equivalent immunoreactivity.

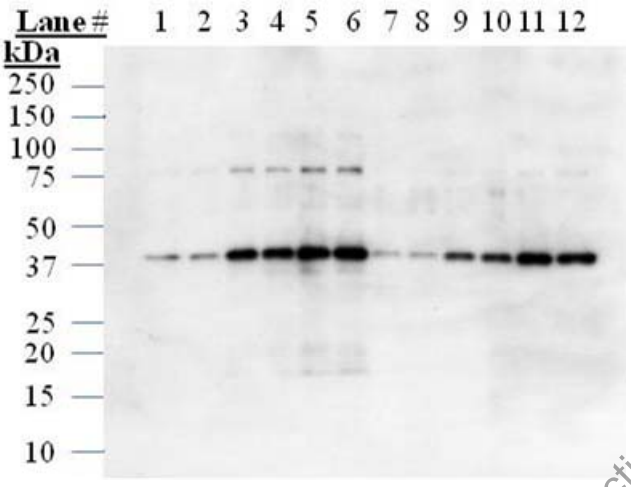

Figure C-3. Western Blot Analysis of MON 88701 DMO and *E. coli*-produced MON 88701 DMO Proteins

Aliquots of the MON 88701 DMO protein and the *E. coli*-produced MON 88701 DMO protein were subjected to SDS-PAGE and electrotransferred to a nitrocellulose membrane. The membrane was incubated with anti-DMO antibodies and immunoreactive bands were visualized using an ECL system (GE Healthcare, Piscataway, NJ). Approximate molecular weights (kDa) are shown on the left. Lanes loaded with molecular weight markers were cropped, and lanes were renumbered relative to the original gel loading. The 6 min exposure is shown. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	*E. coli*-produced MON 88701 DMO protein	0.5
2	*E. coli*-produced MON 88701 DMO protein	0.5
3	*E. coli*-produced MON 88701 DMO protein	2
4	*E. coli*-produced MON 88701 DMO protein	2
5	*E. coli*-produced MON 88701 DMO protein	6
6	*E. coli*-produced MON 88701 DMO protein	6
7	MON 88701 DMO protein	0.5
8	MON 88701 DMO protein	0.5
9	MON 88701 DMO protein	2
10	MON 88701 DMO protein	2
11	MON 88701 DMO protein	6
12	MON 88701 DMO protein	6

Table C-3. Comparison of Immunoreactive Signals Between MON 88701 DMO and *E. coli*-produced MON 88701 DMO Proteins

Mean Signal intensity from MON 88701 DMO ($OD \times mm^2$)	Mean Signal intensity from *E. coli*-produced MON 88701 DMO ($OD \times mm^2$)	Preset Acceptance limits for MON 88701 DMO[1] ($OD \times mm^2$)
4.440	6.500	4.225 – 8.775

[1]The acceptance limits for MON 88701 DMO are based on the interval between +35% (6.500 × 1.35) and -35% (6.500 × 0.65) of the overall mean of the *E. coli*-produced MON 88701 DMO signal intensity across six loads.

C.2.9. Molecular Weight and Purity Estimation using SDS-PAGE

C.2.9.1. Methods

MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were mixed with 5X LB and diluted with water to a final total protein concentration of 0.1 µg/µl. Molecular Weight Standards, Bio-Rad broad range (Hercules, CA) were diluted to a final total protein concentration of 0.9 µg/µl. The MON 88701 DMO was analyzed in duplicate at 0.5, 1, and 1.5 µg protein per lane. The *E. coli*-produced MON 88701 DMO reference standard was analyzed at 0.5 µg total protein in a single lane. The samples were loaded onto a 10-well pre-cast Tris glycine 4-20% (w/v) polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA) and electrophoresis was performed at a constant voltage of 125 V for 90 min. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) acetic acid for 25 min, stained for ~16 h with Brilliant Blue G-Colloidal stain (Sigma-Aldrich, St. Louis, MO). Gels were destained once for 30 sec with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, and with 25% (v/v) methanol for a total of 6 h. 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). The apparent MW of each observed band was estimated from a standard curve generated by the Quantity One software which was based on the MWs of the markers and their migration distance on the gel. To determine purity, all visible bands within each lane were quantified using Quantity One software. Apparent MW and purity were reported as an average of all six lanes containing the MON 88701 DMO.

C.2.9.2. Results of MON 88701 DMO Protein Molecular Weight Equivalence

The molecular weight and purity of the MON 88701 DMO protein were determined to be 39.5 kDa and 97%, respectively. To assess molecular weight (MW) and purity, the MON 88701 DMO protein was subjected to SDS-PAGE. The gel was stained with Brilliant Blue G Colloidal stain and analyzed by densitometry (Figure C-4). *E. coli*-produced MON 88701 DMO protein was loaded in a single lane for reference (Figure C-4, Lane 2). The MON 88701 DMO protein (Figure C-4, Lanes 3-8) had an apparent MW of 39.5 kDa (Table C-4). The apparent MW of the *E. coli*-produced MON 88701 DMO protein as reported on its Certificate of Analysis was 38.7 kDa (Table C-4). Because the apparent MW of MON 88701 DMO protein was within the preset acceptance limits for equivalence (Table C-4), the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were determined to have equivalent apparent MWs.

The purity of the MON 88701 DMO protein was calculated based on the six loads on the gel (Figure C-4, Lanes 3 to 8). The average purity was determined to be 97%.

Table C-4. Molecular Weight Comparison Between the MON 88701 DMO and *E. coli*-produced MON 88701 DMO Proteins Based on SDS-PAGE

Apparent MW of MON 88701 DMO Protein[1] (kDa)	Apparent MW of *E. coli*-Produced MON 88701 DMO Protein[2] (kDa)	Preset Acceptance Limits for MON 88701 DMO[3] (kDa)
39.5	38.7	38.5-39.7

[1]The reported value is the mean molecular weight across all six loads.
[2]The molecular weight of the *E. coli*-produced MON 88701 DMO protein as reported on its Certificate of Analysis.
[3]See Section C.6.

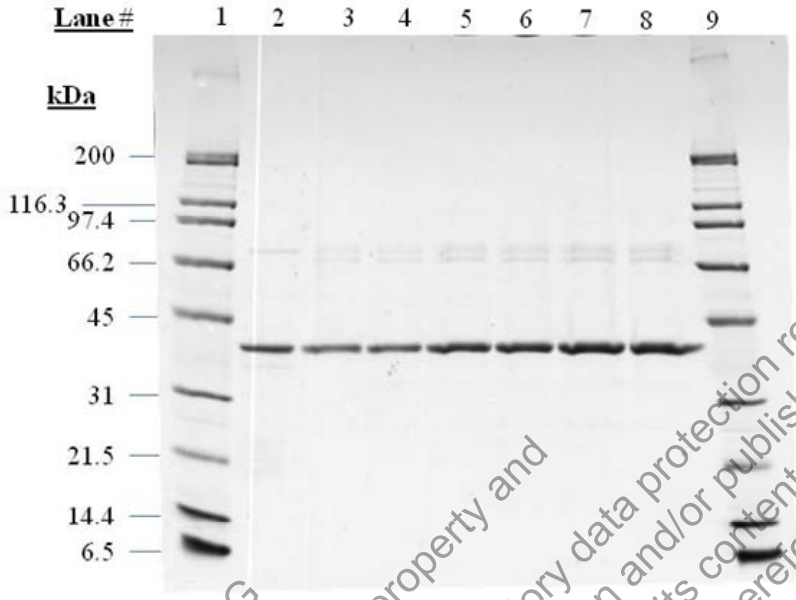

Figure C-4. Molecular Weight and Purity Analysis of the MON 88701 DMO Protein

Aliquots of the MON 88701 DMO and the *E. coli*-produced MON 88701 DMO proteins were separated by SDS-PAGE and then stained with Brilliant Blue G-Colloidal stain. Approximate molecular weights are shown on the left and correspond to the markers loaded in Lanes 1 and 9. Empty lane was partially cropped. Lane designations are as follows:

Lane	Sample	Amount (µg)
1	Broad Range Molecular Weight Markers	4.5
2	*E. coli*-produced MON 88701 DMO protein	0.5
3	MON 88701 DMO protein	0.5
4	MON 88701 DMO protein	0.5
5	MON 88701 DMO protein	1
6	MON 88701 DMO protein	1
7	MON 88701 DMO protein	1.5
8	MON 88701 DMO protein	1.5
9	Broad Range Molecular Weight markers	4.5

C.2.10. Glycosylation Analysis

C.2.10.1. Methods

Glycosylation analysis was used to determine whether the MON 88701 DMO was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 88701 DMO protein, the *E. coli*-produced MON 88701 DMO (negative control) and the positive control, transferrin (Sigma-Aldrich, St Louis, MO), were each diluted with water and brought to 1X LB. These samples were heated at ~101 °C for 3 min. The MON 88701 DMO, the *E. coli*- produced MON 88701 DMO and transferrin were loaded at approximately 50 and 100 ng per lane on a Tris-glycine 10 well 4-20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA). Precision Plus Protein Dual color Standards (Bio-Rad, Hercules, CA) were also loaded to verify electrotransfer of the proteins to the membrane and as markers for molecular weight. Electrophoresis was performed at a constant voltage of 150 V for 90 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 25 V.

Carbohydrate detection was performed directly on the PVDF membrane at room temperature using the Amersham ECL glycoprotein Detection Module (GE, Healthcare, Piscataway, NJ). With this module, carbohydrate moieties of proteins were oxidized with sodium metaperiodate and then biotinylated with biotin-X-hydrazide. The biotinylated proteins can be detected on the blot by addition of streptavidin conjugated to HRP for luminol-based detection using ECL reagents (GE, Healthcare, Piscataway, NJ) and with subsequent exposure to Amersham Hyperfilm (GE, Healthcare). The film was developed using a Konica SRX-101A automated film processor (Konica Minolta Medical & Graphic, Inc., Tokyo, Japan).

An identical blot run in parallel to that used for the glycosylation analysis was stained to visualize the proteins present on the membrane. Proteins were stained for 30 sec to 2 min using Coomassie Brilliant Blue R-250 staining solution (Bio-Rad, Hercules, CA) and then destained with 1X Coomassie Brilliant Blue R-250 destaining solution (Bio-Rad) for 5 min. After washing with water, the blot was dried and scanned using Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0).

C.2.10.2. Results of Glycosylation Analysis

Some eukaryotic proteins are post-translationally modified by the addition of carbohydrate moieties (Rademacher et al., 1988). To test whether DMO protein was glycosylated when expressed in the cottonseed of MON 88701, the MON 88701 DMO protein was analyzed using an ECL Glycoprotein Detection Module (GE, Healthcare, Piscataway, NJ). Transferrin, a glycosylated protein, was used as a positive control in the assay. To assess equivalence of the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins, the *E. coli*-produced MON 88701 DMO protein was also analyzed. The positive control was clearly detected at expected molecular weight (~80 kDa) and the band intensity increased with increasing concentration (Figure C-5, Panel A, Lanes 1-2). In contrast, signals were not observed in the lanes containing the

MON 88701 DMO or *E. coli*-produced MON 88701 DMO proteins at the expected molecular weight for the MON 88701 DMO protein (Figure C-5 Panel A, Lanes 7-8 and Lanes 4-5, respectively). To assess that sufficient MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were present for glycosylation analysis, a second membrane (with identical loadings and transfer times) was stained with Coomassie Blue R250 for protein detection (Figure C-5 Panel B). Both the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were clearly detected (Figure C-5 Panel B, Lanes 7-8 and Lanes 4-5, respectively). These data indicate that the glycosylation status of MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins are equivalent and that neither is glycosylated.

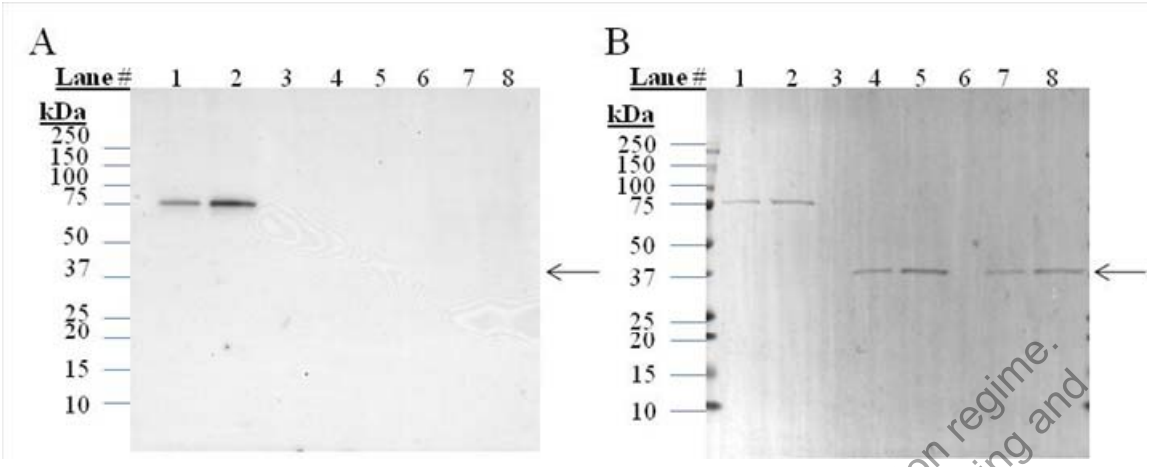

Figure C-5. Glycosylation Analysis of the MON 88701 DMO Protein
Aliquots of the transferrin (positive control), *E. coli*-produced MON 88701 DMO protein and MON 88701 DMO protein were subjected to SDS-PAGE and electrotransferred to PVDF membranes. Panel A corresponds to detection of the labeled carbohydrate moieties, where present, using the ECL-based system with exposure to Hyperfilm. A 6 min exposure is shown. Panel B corresponds to Coomassie Brilliant Blue R250 staining of an equivalent blot to confirm the presence of proteins. The signal was captured using a Bio-Rad GS-800 with Quantity One software (version 4.4.0). Approximate molecular weights (kDa) correspond to the Precision Plus, dual color markers (used to verify transfer and MW). Lanes loaded with molecular weight markers were partially cropped, and lanes were renumbered relative to the original gel loading. Arrows indicate the expected migration MON 88701 DMO protein. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	Transferrin (positive control)	50
2	Transferrin (positive control)	100
3	Empty	-
4	*E. coli*-produced MON 88701 DMO (negative control)	50
5	*E. coli*-produced MON 88701 DMO (negative control)	100
6	Empty	-
7	MON 88701 DMO	50
8	MON 88701 DMO	100

C.2.11. Functional Activity Analysis

C.2.11.1. Methods

The specific activity of MON 88701 DMO and *E. coli*-produced MON 88701 DMO was determined by quantifying the conversion of 3,6-dichloro-2-methoxybenzoic acid (dicamba) to 3,6-dichlorosalicylic acid (DCSA) via HPLC (Agilent Technologies 1100 series, Santa Clara, CA) separation and fluorescence detection (Agilent Technologies 1200 series, G1321A). Each assay reaction contained 25 mM potassium phosphate, pH 7.2, 3.4 µg ferredoxin, 3.4 µg reductase, 0.5 mM $FeSO_4$, 10 mM $MgCl_2$, 0.7 mM NADH, 0.3 mM dicamba, 2 µl (42.48 U/ml) of formaldehyde dehydrogenase and either 2.9 µg MON 88701 DMO or 3 µg his-DMO as an assay positive control. The reactions were performed in PCR tubes (Sorenson, Salt Lake City, UT) and incubated at 30 °C for 15 min. Reactions (200 µl) were initiated by the addition of dicamba and quenched with the addition of 50 µl of 5% H_2SO_4. Reactions were then filtered using Whatman Anotop 10 filters (0.2 µm, GE healthcare), and 40 µl was transferred to a HPLC sample vial (200 µl, Agilent) for analysis. Twenty-five microliters of the filtered reaction was injected onto a Phenomenex® Synergi 4 µm C18/ODS Hydro-RP column (150 × 4.6 mm ID, Torrance, CA). The mobile phase consisted of solvent A (21.5 mM phosphoric acid) and solvent B (100% acetonitrile) running at 1.5 ml/min. DCSA was eluted from the column using a linear gradient from 90% to 40% solvent A for the first 14 min, followed by a step to 10% solvent A for 1 min and then re-equilibration at 90% solvent A for 10 min before the next injection. DCSA was monitored by the detection of fluorescent emission at 424 nm (excitation 306 nm) and quantified relative to a standard curve of DCSA generated using 0.1, 0.3, 0.6, 0.9, 1.2, 2.4, and 4.8 nmol/250 µl. Chromatographic data were collected using Atlas™ 2003 software (Thermo Fisher Scientific Inc). The specific activity was calculated based on the amount of purity corrected MON 88701 DMO protein added to the reaction mixture and expressed as nmol of DCSA produced per minute per mg of MON 88701 DMO protein (nmol × min^{-1} × mg^{-1}).

C.2.11.2. Results of Functional Activity

The functional activities of the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were determined by quantifying the conversion of dicamba to DCSA using HPLC separation and fluorescence detection. In this assay, protein-specific activity is expressed as nmol DCSA × $minute^{-1}$ × mg^{-1} of DMO.

The experimentally-determined specific activities for the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins are presented in Table C-5. The specific activities of MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were 5.48 and 7.23 nmol DCSA × $minute^{-1}$ × mg^{-1} of DMO, respectively. Because the mean specific activities of the MON 88701-produced and *E. coli*-produced MON 88701 DMO proteins fall within the preset acceptance criterion (Table C-5), the MON 88701 DMO and *E. coli*-produced MON 88701 DMO proteins were determined to have equivalent functional activity.

Table C-5. MON 88701 DMO Functional Activity Assay

MON 88701 DMO[1] (nmol DCSA × minute^{-1} × mg^{-1})	*E. coli*-produced MON 88701 DMO[1] (nmol DCSA × minute^{-1} × mg^{-1})	Preset Acceptance Limits for MON 88701 DMO[2] (nmol DCSA × minute^{-1} × mg^{-1})
5.48 ± 1.3	7.23 ± 2.1	1.69-20.74

[1]Value refers to mean and standard deviation calculated based on n = 5.
[2]See Section C.6.

C.3. Substrate Specificity of MON 88701 DMO Protein

C.3.1. Exogenous Specificity Herbicide Tolerance - Greenhouse Analysis

C.3.1.1. Materials

MON 88701 (lots IG200000043964508005990 4 and IG200000037145900213862 4) and the near isogenic conventional control, Coker 130, (lots IG200000002572639259852 8, IG200000002572640754073 6, and IG200000002572637293772 8) were grown in greenhouses during 2010 and 2011. At the 2-5 leaf growth stage or pre-emergent, MON 88701 and the near isogenic conventional control, Coker 130, were sprayed with different herbicides. The herbicides tested are listed in Table C-6.

Table C-6. Herbicides Tested in Exogenous Specificity Herbicide Tolerance Greenhouse Trials

Manufacturer/ Retailer	Herbicide	Herbicide Formulation	Lot Number
Albaugh	2,4-DB	Butyrac® 200	HPR-0404-14987-F
BASF	dicamba	Clarity®	KIH-0702-18134-F
Dow	atrazine	Atrazine	AGT-0804-19336-F
Dow	trifluralin	Treflan®	MB231656T7
Dow	oxyfluorfen	Goal® 2XL	EWP-0107-11628-F
Helena	2,4-D	2,4-D Amine 4	RUD-0502-15805-F
Monsanto	acetochlor	Harness®	MUS-0704-18520-F
Monsanto	halosulfuron	Permit®	MUS-0405-15154-F
Monsanto	glyphosate	Roundup WeatherMax®	MUS-0905-19887-F
Syngenta	paraquat	Gramoxone®	GTA-0606-17421

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

C.3.1.2. Exogenous Specificity Herbicide Tolerance Greenhouse Method

MON 88701 and the near isogenic conventional control, Coker 130, were planted in pots containing Redi-earth® and Osmocote® 14-14-14 slow release fertilizer or Peters® 20-20-20 fertilizer. There were 10 replicate pots and one plant per replicate of MON 88701 and the conventional control for each herbicide and rate tested. The pots were randomly placed in a greenhouse and grown under normal agronomic conditions for cotton (relative humidity 10-70%, temperature 21-34˚C, 14 hour photoperiod, and watering as needed). At pre-emergence or when the plants were at the 2-5 leaf growth stage, the replicates were treated with a single herbicide and rate (Table 1). Two different application rates of each herbicide were applied to different replicate sets (Table 2). Based on the U.S. herbicide labeled rates, the rates for the experiments were chosen and then adjusted for use on cotton and for the optimal growing conditions in the greenhouse in order to achieve approximately 40 to 80% injury. Twenty to 22 days after application, all plants were rated for percent injury. Ratings were based on visual assessment of chlorosis, necrosis, malformation, stunting, and biomass reduction with 0 being no visible injury and 100 being completely dead. All 10 replicate ratings were averaged.

C.3.1.3. Results of Herbicide Tolerance Greenhouse Trials

MON 88701 demonstrated reduced injury ratings for dicamba, but similar injury ratings, and therefore similar levels of susceptibility as the near isogenic conventional control, Coker 130, for the remaining 9 herbicides tested (Table C-7).

Table C-7. Herbicide Tolerance Trials Injury Ratings

Formulation	Manufacturer	Herbicide[1]	Labeled Rate Range (g/ha)[2]	Rates Applied (g/ha)[2]	Injury Observations (days after application)	Injury ratings (%)[3] Control[4] Average (Range)	Injury ratings (%)[3] MON 88701[5] Average (Range)
Clarity®	BASF	dicamba	140-2242 (a.e.)	561 (a.e.) 1120 (a.e.)	22	87 (80-90) 92 (85-95)	0 5 (3-8)
2,4-D Amine 4	Helena	2,4-D	140-2242 (a.e.)	280 (a.e.) 561 (a.e.)	22	86 (80-90) 96 (90-99)	88 (85-95) 98 (95-99)
Butyrac® 200	Albaugh	2,4-DB	130-1682 (a.e.)	280 (a.e.) 561 (a.e.)	22	79 (70-85) 96 (90-99)	84 (75-95) 96 (90-99)
Gramoxone®	Syngenta	paraquat	280-1120 (a.e.)	561 (a.e.) 841 (a.e.)	20	88 (85-95) 95 (90-98)	84 (80-90) 95 (90-100)
Harness®	Monsanto	acetochlor	930-4485 (a.i.)	4485 (a.i.) 6732 (a.i.)	21	70 (30-100) 84 (40-100)	68 (50-100) 93 (80-100)
Atrazine	Dow	atrazine	1100-3800 (a.i.)	1682 (a.i.) 3364 (a.i.)	21	28 (20-40) 48 (20-95)	29 (20-40) 62 (20-100)
Treflan®	Dow	trifluralin	560-2242 (a.i.)	4485 (a.i.) 6732 (a.i.)	21	2 (0-5) 4 (0-10)	1 (0-5) 5 (0-10)
Roundup WeatherMax®	Monsanto	glyphosate	280-4162 (a.e.)	75 (a.e.) 240 (a.e.)	21	9 (5-20) 46 (35-60)	12 (5-25) 50 (40-60)
Goal® 2XL	Dow	oxyfluorfen	280-2242 (a.i.)	561 (a.i.) 841 (a.i.)	21	37 (25-25) 46 (30-80)	41 (30-50) 46 (35-80)
Permit®	Monsanto	halosulfuron	36-140 (a.i.)	75 (a.i.) 200 (a.i.)	21	48 (40-55) 59 (50-65)	50 (45-55) 59 (55-65)

[1]Herbicides applied pre-emergent were acetochlor, atrazine, and trifluralin. All other herbicides were applied when the plants were at the 2-5 leaf growth stage.
[2]a.e. = acid equivalent; a.i. = active ingredient. Each herbicide contains the active ingredient directly or the salt form of the active ingredient. When determining the rate of application, the salt form is calculated back to the acid that is the active ingredient and therefore called acid equivalent. Each labeled rate is for cereal or/and broad acre row crops since these herbicides are not labeled to be sprayed on cotton or are labeled for cotton only as a pre-plant treatment. Based on the labeled rates, the rates for the experiments were chosen and then adjusted for use in-crop on cotton and for the optimal growing conditions in the greenhouse.
[3]Injury ratings were determined by visual inspection of each plant. Ratings were based on visual assessment of chlorosis, necrosis, malformation, stunting, and biomass reduction. 0 percent = no visual adverse effects and 100 percent = completely dead.
[4]Control plants were near isogenic conventional cotton control Coker 130. Reported average and range of 10 replicate plants.
[5]Reported average and range of 10 replicate plants.

C.3.2. *In Vitro* Endogenous Specificity Experiments

C.3.2.1 Materials

The DMO protein used in the endogenous specificity *in vitro* experiments was generated in *Escherichia coli* with a histidine-tag at the N-terminus and has an identical amino acid sequence to MON 88701 DMO with the exception of the lack of leucine at the second position and the nine amino acids from CTP2 (Figure C-1). The compounds tested and standards used in the *in vitro* experiments are listed in Table C-8.

Table C-8. Compounds Used in Specificity *In Vitro* Experiments

Manufacturer/ Retailer	Compound	Common Name	Lot/Product Number
Compounds Tested:			
Aldrich	2-methoxybenzoic acid	o-anisic acid	A0230443
Chem Service	3,6-dichloro-2-methoxybenzoic acid	dicamba	341-9143
Sigma	2,4-dichlorophenoxyacetic acid	2,4-D	D7299-100G
Sigma	3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid	sinapic acid	D7927-1G
Fluka	3,5-dimethoxy-4-hydroxybenzoic acid	syringic acid	86230
Fluka	4-hydroxy-3-methoxybenzoic acid	vanillic acid	94770
Fluka	3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid	ferulic acid	46278
Compounds Used as Standards:			
Monsanto	3,6-dichlorosalicylic acid	DCSA	GLP-0603-16959-T
Riedel-de Haen	2,4-dichlorophenol	2,4-DCP	35811

C.3.2.2. *In Vitro* Specificity Experiments Enzymatic Reaction Mixture Method

The reaction of *E. coli*-produced DMO with different compounds evaluated as potential substrates was carried out using similar reaction conditions described in the characterization portion of this appendix (Appendix C.2.10.). The compounds (Table C-8) were combined with *E. coli*-produced DMO at 0.2 and/or 0.012 mM. The concentrations tested ensured adequate reaction conditions in terms of the substrate for the detection of product formation or disappearance of substrate.

C.3.2.3. *In Vitro* Experiments Liquid Chromatography Separation Method

The reaction mixture was separated by Ultra Performance Liquid Chromatography (UPLC) using an ACQUITY UPLC BEH C18 Column containing 1.7 µm Bridged Ethyl Hybrid (BEH) particles and an ACQUITY BEH C18 VanGuard Pre-column. The

column was heated to 40°C. The tested substrates and potential oxidative by-products were monitored by ACQUITY UPLC photodiode array (PDA) with wavelength range from 200nm to 320nm with 1.2nm resolution. The chromatography was performed at 0.25ml/min and directed to the mass spectrometer following the separation. Both mobile phase A (water) and solvent B (acetonitrile) contained 0.1% v/v formic acid. Gradients used were substrate-specific:

- The gradient for dicamba was run from 40 to 50% solvent B in 3min, 50 to 100% solvent B in 0.1 min and then kept at 100% solvent B for 1min before returning to 40% solvent B in 0.1 min.

- The gradient for 2,4-D was run from 40 to 45% solvent B in 6min, held at 45% solvent B for 1min, 45 to 100% solvent B in 0.1 min, and then held at 100% solvent B for 0.5 min before returning to 40% solvent B in 0.1 min.

- The gradient for ferulic acid, o-anisic acid, sinapic acid, syringic acid, and vanillic acid were run from 0 to 100% solvent B in 4 min and then held at 100% solvent B for 1 min before returning to 0% solvent B in 0.1 min.

Five microliters injection of each sample was used for UPLC analysis where the disappearance of the potential substrate was monitored, and a 50 µl injection was used for UPLC analysis where formation of potential oxidative by-products was monitored.

C.3.2.4. *In Vitro* Experiments Mass Spectrometry Detection Method

Elution from the UPLC column (C.3.2.3) flowed directly to a Waters Micro Q-TOF mass spectrometer. The parameters used for the mass determination were: negative mode, capillary voltage of 2800 V, sample cone voltage of 26 V for all analytes with the exception of 2,4-D and 2,4-DCP, which was 10 V. The extraction cone was 1.5 V. The source temperature was 150 °C and the desolvation temperature was 390 °C. The desolvation gas flow was 500 L/hour. Scan time was 0.76 seconds and inter-scan delay was 0.1 seconds. The m/z range used was specific to each substrate and product. The m/z range for dicamba and DCSA was from 160 to 225 from 0 to 4 minutes. The m/z at 175, which is the fragment ion of dicamba, was used as a detection method for dicamba. This fragment ion of dicamba gave better sensitivity than the parent ion. The m/z at 205 or 207 was used to detect DCSA. The m/z range for 2,4-D and 2,4-DCP was from 160-164 or 160-225, dependent on the specific experiment from 0 to 6 minutes. The m/z range for all other acids is from 120 to 230 within 4 minutes.

C.3.2.5. Results of *In Vitro* Experiments with Endogenous Cotton Compounds

The reaction of dicamba with *E. coli*-produced DMO has been well characterized utilizing an *in vitro* enzymatic assay that monitors the formation of DCSA by LC-MS, which allows for the detection of the product with high sensitivity. Both the substrate and reaction products can be detected by LC-UV and LC-MS after separation by UPLC (Figure C-6).

Compounds structurally similar to dicamba and found in cotton, soybean and corn were used as potential substrates to determine if these compounds could be metabolized by DMO (Table C-8). The compounds tested were syringic acid, o-anisic acid, vanillic acid, ferulic acid, and sinapic acid. Mass spectrometry scans were taken from 120 m/z to 250 m/z to cover the range of all potential oxidation products formed by DMO. Standard reaction conditions of dicamba with a histidine tagged *E. coli*-produced DMO were used as a positive control. LC-MS data demonstrated that there are no additional peaks formed when reactions of each compound incubated with histidine tagged *E. coli*-produced DMO and without histidine tagged *E. coli*-produced DMO are compared (Figure C-7) (dicamba m/z 205, 2, 4-D m/z 163, ferulic acid m/z 175, o-anisic acid m/z 137, sinapic acid m/z 209, syringic acid m/z 183, and vanillic acid m/z 153). There were no peaks observed at the respective masses for the predicted reaction products of each compound incubated with histidine tagged *E. coli*-produced DMO, indicating these compounds are not catabolized by DMO.

To assess whether MON 88701 DMO protein has the same specificity as the histidine tagged DMO used in the *in vitro* experiments, the *E. coli*-produced MON 88701 DMO protein (*i.e.*, lacking a histidine tag), shown to be equivalent to the plant produced MON 88701 DMO protein (Section V.B), was incubated with *o*-anisic acid, the endogenous compound that has the greatest structural similarity to dicamba. Again dicamba was used as a positive control to demonstrate the assay system was functional (Figure C-8). This analysis demonstrated that *o*-anisic acid was not metabolized by the *E. coli*-produced MON 88701 DMO protein (*i.e.*, lacking a histidine tag), but dicamba was (Figures C-8 and C-9). These results indicate that DMO, including the MON 88701 DMO protein, is specific for dicamba as a substrate.

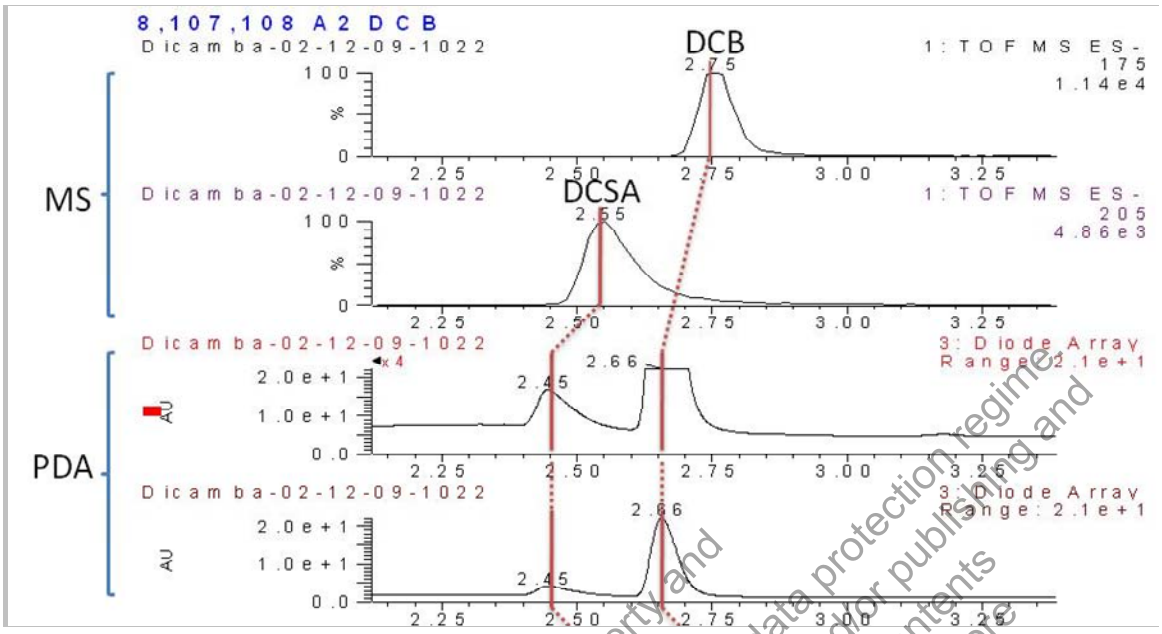

Figure C-6. UPLC Separation of Dicamba (DCB) and DCSA in Five Substrate Analysis

Dicamba and DCSA were separated by UPLC and detected by UV absorbance using a Photo Diode Array (PDA) and mass spectrometry (MS).

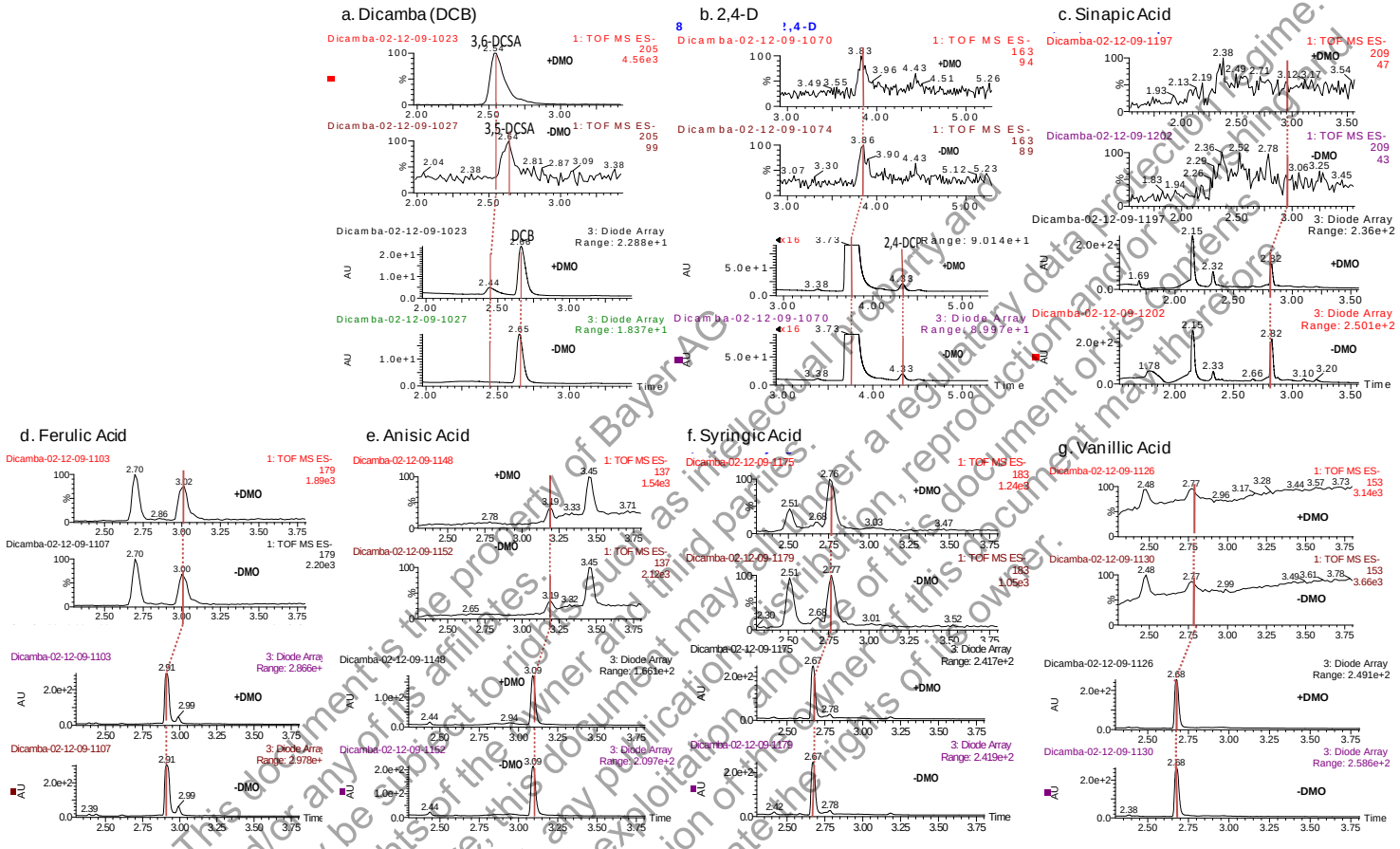

Figure C-7. *E. coli*-produced DMO Conversion of Endogenous Substrates

Endogenous substrates, as well as dicamba and 2,4-D, were incubated with *E. coli*-produced DMO and the formation of products and disappearance of substrate was monitored by LC-MS (top two chromatograms) and LC-UV (bottom two chromatograms) for a positive control (dicamba (a)), (2,4-D (b)) and each endogenous compound:, sinapic acid(c), ferulic acid (d), anisic acid (e), syringic acid (f), and vanillic acid (g). For each experiment the reaction mixture was made with (+*E. coli*-produced DMO, upper) and without (-*E. coli*-produced DMO, lower). The red line indicates the migration of the substrates (and DCSA in the case of dicamba) in each chromatogram.

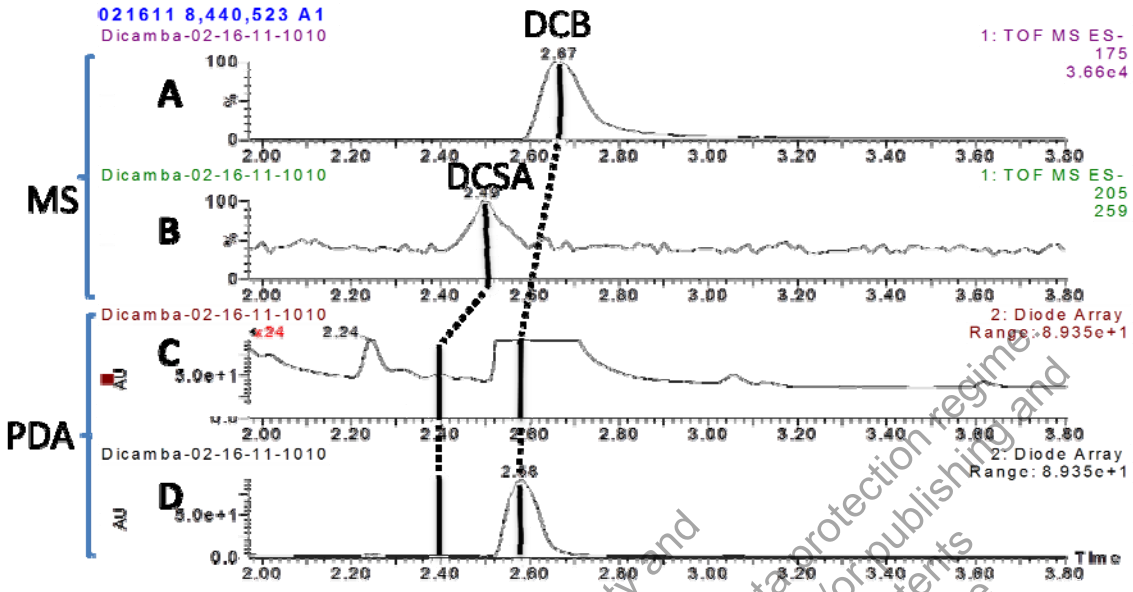

Figure C-8. UPLC Separation of Dicamba (DCB) and DCSA in Bridging Analysis
Dicamba and DCSA were separated by UPLC and detected by UV absorbance using a Photo Diode Array (PDA) and mass spectrometry (MS).

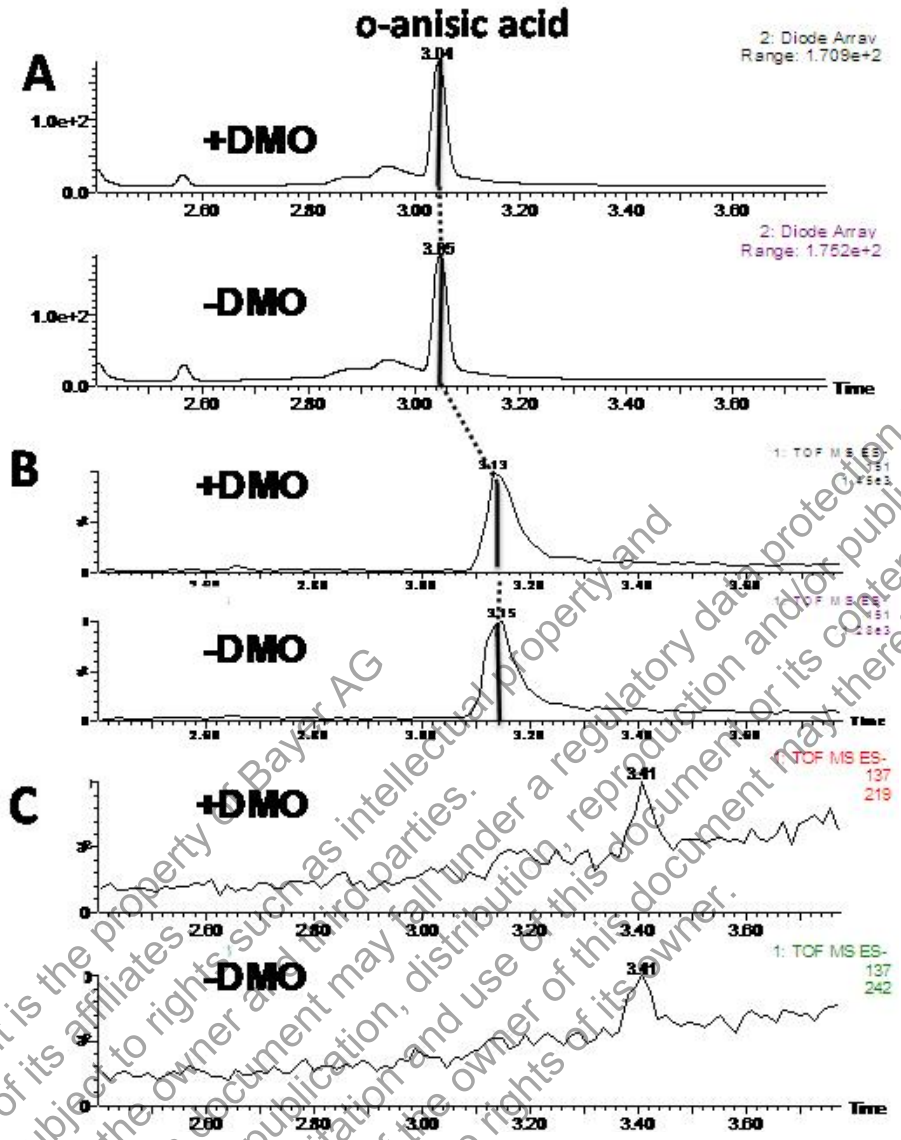

Figure C-9. *E. coli*-produced MON 88701 DMO Conversion of *o*-Anisic Acid

o-anisic acid was incubated with *E. coli*-produced MON 88701 DMO and the formation of predicted oxidative product and the disappearance of *o*-anisic acid was monitored by LC-UV (A chromatograms) and LC-MS (B and C chromatograms). *o*-Anisic acid was included in a reaction mixture made with (+DMO, upper) and without (-DMO, lower) MON 88701 DMO. The dotted line indicates the solvent delay between the UV and MS detectors as they are connected in series.

C.4. Characterization of PAT (*bar*) Protein in MON 88701

C.4.1. Materials

The MON 88701-produced PAT (*bar*) protein (lot 11295997) was purified from cottonseed of MON 88701 (lot 11287350). The MON 88701-produced PAT (*bar*) protein was stored in a -80 ºC freezer in a buffer solution containing 50 mM Tris-HCl, pH 7.5, 0.16 M sodium chloride and 20% glycerol.

The *E. coli*-produced PAT (*bar*) protein (lot 11270310) was used as the reference substance. The PAT (*bar*) protein reference substance was generated from cell paste produced by large-scale fermentation of *E. coli* containing the pMON106653 expression plasmid. The coding sequence for *bar* contained on the expression plasmid (pMON106653) was confirmed prior to and after fermentation. The *E. coli*-produced PAT (*bar*) protein was previously characterized.

C.4.2. Description of Assay Control

Protein MW standards (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were used to calibrate some SDS-PAGE gels and verify protein transfer to polyvinylidene difluoride (PVDF) and nitrocellulose membranes. Broad Range SDS-PAGE molecular weight standards (Bio-Rad, Hercules, CA) were used to generate a standard curve for the apparent MW estimation. Bovine serum albumin (BSA) and α-aminobutyric acid (AAbA) were used as hydrolysis control and internal calibration standard for amino acid analysis. A phenylthiohydantoin (PTH) amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to calibrate the Applied Biosystems 494 Procise Sequencing System for each analysis. A peptide mixture (Sequazyme Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. Transferrin (Sigma-Aldrich, St. Louis, MO) was used as positive control for glycosylation analysis.

C.4.3. PAT (*bar*) Protein Purification

The plant-produced PAT (*bar*) protein was purified from cottonseed of MON 88701. The purification procedure was not performed under a GLP plan; however, all procedures were documented on worksheets and, where applicable, SOPs were followed. The plant-produced PAT (*bar*) was purified from an extract of ground cottonseed using a combination of dye affinity chromatography and anionic exchange chromatography. The purification procedure is briefly described below.

Approximately 1 kg of cottonseed of MON 88701 was ground to fine powder using a laboratory mill (model 3100, Perten Instruments). The ground powder was suspended in 4 liters of hexane (EMD Chemicals Inc., Gibbstown, NJ) and filtered 3 times in order to defat the powder. After drying overnight, the powder was ready for further processing. All grinding and defatting steps were done in a fume hood at room temperature.

A portion (200 g) of the defatted powder was extracted with 2 liters of 20 mM Tris-HCl, pH 7.5, and the solids were removed by centrifugation at 25,000 × g for 20 min. The decanted solution was treated with 15 ml of 1 M CaCl2 solution to precipitate some proteins and centrifuged at 25,000 × g for 20 min to remove the precipitated proteins. The soluble portion (~1450 ml), containing the PAT (*bar*) protein, was batch absorbed onto 20 ml of reactive brown 10 agarose (Sigma-Aldrich, St. Louis, MO) equilibrated with 200 ml of 20 mM Tris-HCl, pH 7.5. The reactive brown 10 agarose was centrifuged at 1000 × g for 2 min and the resin, after decanting the supernatant, was transferred to a column. To remove unbound proteins, reactive brown 10 agarose was washed with 80 ml of 20 mM Tris-HCl buffer, pH 7.5, followed by 120 ml of 20 mM Tris-HCl buffer, pH 7.5, 1.5 M NaCl. Finally, the column was rinsed with 120 ml of 20 mM Tris-HCl buffer, pH 7.5. The PAT (*bar*) protein was then eluted, with 80 ml of 1 mM acetyl CoA in 20 mM Tris-HCl, pH 7.5. The eluted PAT (*bar*) protein was loaded onto a 1 ml Q Sepharose Fast Flow (GE Healthcare) column, equilibrated with 10 ml of 20 mM Tris-HCl, pH 7.5, using an automated chromatography system (AKTA, GE Healthcare). The Q Sepharose Fast Flow column was washed with 20 ml of 20 mM Tris-HCl, pH 7.5, 0.1 M NaCl and consecutive step wise elution using 0.2 M and 0.5 M NaCl in 20 mM Tris-HCl, pH 7.5 to a total volume of 23 ml was conducted. Fractions containing PAT (*bar*) protein were pooled (8 ml) and concentrated to a volume of 1170 µl using a centrifugal filter (Ultracel 10K; Millipore, Billerica, MA; Molecular Weight Cutoff (MWCO) of 10 kDa). Buffer was added to the concentrated sample to bring the final volume to 2 ml and the final buffer composition to 50 mM Tris-HCl, pH 7.5, 0.16 M NaCl, and 20% (v/v) glycerol. This PAT (*bar*) protein purified from the cottonseed of MON 88701 was aliquoted and stored in a -80 °C freezer.

C.4.4. Summary of PAT (*bar*) Protein Identity and Equivalence

Table C-9. Summary of MON 88701-produced PAT (*bar*) Protein Identity and Equivalence

Analytical Test Assessment	Section Cross Reference	Analytical Test Outcome
1. N-terminal sequence analysis of the MON 88701-produced PAT (*bar*) protein to assess identity	VI.C.3.1.	• The identity was confirmed by N-terminal sequence analysis
2. MALDI-TOF MS[1] analysis of peptides derived from tryptic digested MON 88701-produced PAT (*bar*) protein to assess identity	VI.C.3.2.	• MALDI-TOF MS[1] analysis yielded peptide masses consistent with the expected peptide masses from the theoretical trypsin digest of the MON 88701 PAT (*bar*) sequence
3. Western blot analysis using anti-PAT (*bar*) polyclonal antibodies to assess identity and immunoreactive equivalence between MON 88701- and the *E. coli*-produced PAT (*bar*) proteins	VI.C.3.3.	• MON 88701-produced PAT (*bar*) protein identity was confirmed using a western blot probed with antibodies specific for PAT protein • Immunoreactive properties of the MON 88701- and the *E. coli*-produced PAT (*bar*) proteins were shown to be equivalent
4. SDS-PAGE[2] to assess equivalence of the apparent molecular weight between MON 88701- and the *E. coli*-produced PAT (*bar*) proteins	VI.C.3.4.	• Electrophoretic mobility and apparent molecular weight of the MON 88701- and the *E. coli*-produced PAT (*bar*) proteins were shown to be equivalent
5. Glycosylation analysis of the PAT (*bar*) protein to assess equivalence between the MON 88701- and the *E. coli*-produced PAT (*bar*) proteins	VI.C.3.5.	• Glycosylation status of MON 88701- and the *E. coli*-produced PAT (*bar*) proteins were shown to be equivalent
6. PAT (*bar*) enzymatic activity analysis to assess functional equivalence between MON 88701- and the *E. coli*-produced PAT (*bar*) proteins	VI.C.3.6.	• Functional activity of the MON 88701- and the *E. coli*-produced PAT (*bar*) proteins were shown to be equivalent

[1] MALDI-TOF MS = Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

[2] SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis

C.4.5. N-Terminal Sequencing

C.4.5.1. Methods

N-terminal sequencing, carried out by automated Edman degradation chemistry, was used to confirm the identity of the MON 88701-produced PAT (*bar*).

One aliquot of MON 88701-produced PAT (*bar*) was used for N-terminal sequence analysis. The analysis was performed for 15 cycles. using automated Edman degradation chemistry (Hunkapiller et al., 1983). An Applied Biosystems 494 Procise Sequencing System equipped with 140C Microgradient system a Perkin Elmer Series 200 UV/VIS Absorbance Detector with Procise™ Control Software (version 2.1) was used for amino acid detection after each cycle. Chromatographic data were collected using SequencePro (version 2.1) software. A control protein, β-lactoglobulin, (Applied Biosystems, Foster City, CA) was analyzed before and after the sequence analysis of the MON 88701-produced PAT (*bar*) protein to verify that the sequencer met performance criteria for repetitive yield and sequence identity. Identity was established if ≥ 8 amino acids, consistent with the predicted sequence of the N-terminus of the MON 88701-produced PAT (*bar*), were observed during analysis.

C.4.5.2. Results of the N-terminal Sequence Analysis

N-terminal sequencing of the first 15 amino acids was performed on MON 88701-produced PAT (*bar*). The expected sequence for the PAT (*bar*) protein deduced from the *bar* gene present in MON 88701 was observed. The data obtained correspond to the deduced PAT (*bar*) protein beginning at amino acid positions 2 and 3 (Table C-10, Experimental Sequence 1 and 2, respectively). The N-terminal methionine residue in the PAT (*bar*) protein was not observed. This result is expected as removal of the N-terminal methionine, catalyzed by methionine aminopeptidase, is common in many organisms and has no effect on protein structure or activity (Arfin and Bradshaw, 1988; Bradshaw et al., 1998; Polevoda and Sherman, 2000). Hence, the sequence information confirms the identity of the PAT (*bar*) protein isolated from the cottonseed of MON 88701.

Amino acid residue # from the N-terminus	→	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Expected Sequence	→	M	S	P	E	R	R	P	A	D	I	R	R	A	T	E	A
			|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Experimental Sequence 1	→	-	S	P	E	R	R	P	A	D	I	R	R	A	T	E	A
					|	|	|	|	|	|	|	|	|	|	|	|	|
Experimental Sequence 2	→	-	-	P	E	R	X	X	A	D	I	X	X	X	T	E	-

Table C-10. N-Terminal Sequence of the MON 88701-produced PAT (*bar*) Protein
The expected amino acid sequence of the N-terminus of PAT (*bar*) protein was deduced from the *bar* coding region present in MON 88701. The experimental sequences obtained from the MON 88701-produced PAT (*bar*) protein were compared to the expected sequence. The single letter IUPAC-IUB amino acid code is M, methionine; S, serine; P, proline; E, glutamic acid; R, arginine; A, alanine; D, aspatic acid; I, isoleucine; and T, threonine. X indicates that the residue was not identifiable; (-) indicates the residue was not observed.

C.4.6. MALDI-TOF Tryptic Mass Map Analysis

C.4.6.1. Methods

MALDI-TOF tryptic mass fingerprint analysis was used to confirm the identity of the MON 88701-produced PAT (*bar*) protein. MON 88701-produced PAT (*bar*) protein was subjected to SDS-PAGE and the gel was stained using Brilliant Blue G Colloidal stain. Each ~25 kDa band was excised and transferred to a microcentrifuge tube. The gel slices were destained with 40% (v/v) methanol/ 10% (v/v) acetic acid and washed in 100 mM ammonium bicarbonate and then, to reduce the protein in each, gel slices were incubated in 100 µl of 10 mM DTT at ~37 °C for 1 h. The protein was then alkylated in the dark for 20 min with 100 µl of 20 mM iodoacetic acid and washed three times for 15-20 min each with 200 µl of 25 mM ammonium bicarbonate. Gel slices were dried with a Speed-Vac® concentrator (Thermo Fisher Scientific, Waltham, MA) and then rehydrated with 20 µl of trypsin solution (20 µg/ml). After 1.25 h, excess liquid was removed and the gel was incubated overnight at ~37.5 °C in 40 µl of 10% acetonitrile in 25 mM ammonium bicarbonate. Gel slices were sonicated for 5 min to further elute proteolytic fragments. The resulting extracts were transferred to new microcentrifuge tubes labeled Extract 1 and dried using Speed-Vac concentrator. The gel slices were re-extracted twice with 30 µl of a 60% acetonitrile, 0.1% trifluoroacetic acid, 0.1% β-octylglucopyranoside solution and sonicated for 5 min. Both extracts were pooled into a new tube labeled Extract 2 and dried with a Speed-Vac concentrator. A solution (20 µl) of 0.1% trifluoroacetic acid (TFA) was added to all Extract 1 and 2 tubes and the samples were dried to completion via vacuum centrifugation. To solubilize the extracts, 5 µl of 50% acetonitrile, 0.1% trifluoroacetic acid was added to Extract 1 tube and 10 µl of 50% acetonitrile, 0.1% trifluoroacetic acid was added to Extract 2 tube and all were sonicated for 5 min. Each extract was spotted to three wells on an analysis plate. For each extract, 0.75 µl of 2, 5 dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (α-Cyano), or 3, 5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) (Thermo Fisher Scientific Inc.) was added to one of the spots. The samples in DHB matrix were analyzed in the 300 to 5000 Da range. Samples in α-Cyano and sinapinic acid were analyzed in the 500 to 5000 Da and 500 to 7500 Da range, respectively. The analysis was performed using a Voyager™ DE Pro Biospectrometry™ workstation (Applied Biosystems) using Voyager Instrument Control Panel software (version 5.10.2) and Data Explorer data analysis software (version 4.0.0.0). Protonated peptide masses were monoisotopically resolved 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 (Lighthouse Data, Odense M, Denmark) was used to generate a theoretical trypsin digest of the PAT (*bar*) protein sequence. Masses within 1 Da of a monosiotopic mass were matched against the theoretical digest of the PAT (*bar*) sequence. All matching masses were tallied and a coverage map was generated for the mass fingerprint. The tryptic mass fingerprint coverage was considered acceptable if ≥ 40% of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments (Biron et al., 2006, Krause et al., 1999).

C.4.6.2. Results of MALDI-TOF Tryptic Mass Map Analysis

The identity of the MON 88701-produced PAT (*bar*) protein was also confirmed by MALDI-TOF MS analysis of peptide fragments produced from tryptic digestion of the MON 88701-produced PAT (*bar*) protein. The ability to identify a protein using this method is dependent upon matching a sufficient number of observed tryptic peptide fragment masses with predicted tryptic peptide fragment masses. In general, protein identification made by peptide mapping is considered to be reliable if ≥ 40% of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments (Biron et al., 2006, Krause et al., 1999).

There were 10 unique peptides identified that corresponded to the masses expected to be produced by tryptic digestion of the PAT (*bar*) protein (Table C-11). The identified masses were used to assemble a coverage map of the entire MON 88701-produced PAT (*bar*) protein (Figure C-10). The experimentally determined mass coverage of the MON 88701-produced PAT (*bar*) protein was 84.7% (155 out of 183 amino acids). This analysis serves as additional identity confirmation for the MON 88701-produced PAT (*bar*) protein.

Table C-11. Summary of the Tryptic Masses[1] Identified for the MON 88701-produced PAT (*bar*) Protein Using MALDI-TOF MS

α-cyano		DHB		Sinapinic acid		Expected Mass	Diff.[2]	Fragment[3]	Sequence
Extract 1	Extract 2	Extract 1	Extract 2	Extract 1	Extract 2				
		879.65				879.46	0.19	113-120	SLEAQGFK
1144.65	1144.75	1144.84				1144.56	0.09	136-145	MHEALGYAPR
1403.93	1404.03	1404.12	1404.18			1403.79	0.14	100-112	TGLGSTLYTHLLK
1523.02	1523.13	1523.14	1523.19	1522.93		1522.86	0.16	121-135	SVVAVIGLPNDPSVR
1843.07	1843.18	1843.27		1842.98	1843.19	1842.85	0.22	38-52	TEPQEPQEWTDDLVR
1859.06	1859.22	1859.22		1858.98	1859.18	1858.86	0.20	81-96	NAYDWTAESTVYVSPR
				2391.45	2391.64	2391.20	0.25	57-78	YPWLVAEVDGEVAGIAYAGPWK
2676.67				2676.64	2676.88	2676.35	0.32	55-78	ERYPWLVAEVDGEVAGIAYAGPWK
				2840.62		2840.32	0.30	13-37	ATEADMPAVCTIVNHYIETSTVNFR
3353.14	3353.36			3353.17	3353.48	3352.73	0.41	155-183	HGNWHDVGFWQLDFSLPVPPRPVLPVTEI

[1]Only experimental masses that matched expected masses are listed in the table.
[2]The difference between the expected mass and the first column mass. Other masses shown within a row are also within 1 Da of the expected mass.
[3]Position refers to amino acid residues within the predicted PAT (*bar*) sequence as depicted in Figure C-10.
DHB = 5-dihydroxybenzoic acid matrix, α-cyano = α-cyano-4-hydroxycinnamic acid matrix, Sinapinic acid = 3, 5-dimethoxy-4-hydroxycinnamic acid matrix

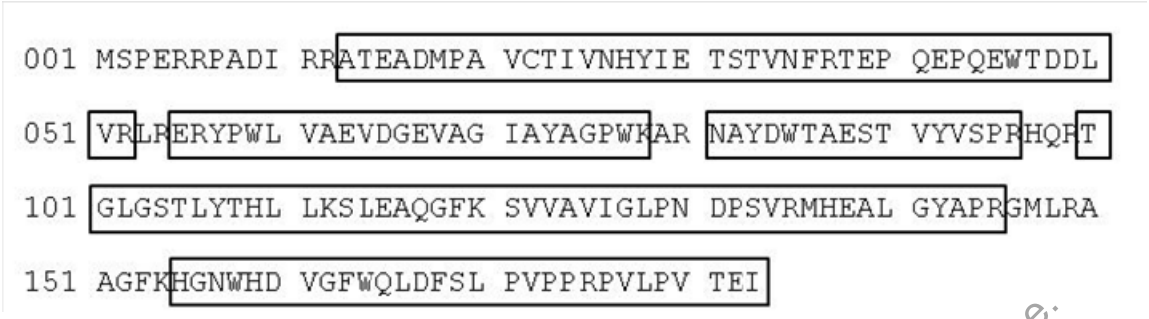

Figure C-10. MALDI-TOF MS Coverage Map of the MON 88701-produced PAT (*bar*) Protein

The amino acid sequence of the PAT (*bar*) protein was deduced from the *bar* gene present in MON 88701. Boxed regions correspond to regions covered by tryptic peptides that were identified from the MON 88701-produced PAT (*bar*) protein sample using MALDI-TOF MS. In total, 84.7% (155 out of 183 amino acids) of the expected protein sequence was covered by the identified peptides.

C.4.7. Western Blot Analysis-Immunoreactivity

C.4.7.1. Methods

Western blot analysis was performed to confirm the identity of the PAT (*bar*) protein purified from cottonseed of MON 88701 and to compare the immunoreactivity of the MON 88701- and *E. coli*-produced proteins.

The MON 88701- and *E. coli*-produced PAT (*bar*) proteins were analyzed concurrently on the same gel using three loadings of 2, 4, and 6 ng. Loadings of the three concentrations were made in duplicate on the gel. Aliquots of each protein were diluted in water and 5X Laemmli buffer (LB) containing 312 mM Tris-HCl, 25% (v/v) 2-mercaptoethanol, 10% (w/v) SDS, 0.025% (w/v) bromophenol blue, 50% (v/v) glycerol, pH 6.8, heated at ~96 °C for 4 min, and applied to a 15 well pre-cast Tris-glycine 4-20% polyacrylamide gradient gel (Invitrogen, Carlsbad, CA). Pre-stained moelcular weight markers (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were loaded in parallel to verify electrotransfer of the proteins to the membrane and to estimate the size of the immunoreactive bands observed. Electrophoresis was performed at a constant voltage of 150 V for 85 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 60 min at a constant current of 200 mA. After electrotransfer, the membrane was blocked for 1 h with 10% (w/v) non-fat dried milk (NFDM) in 1X phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBST). The membrane was then probed with a 1:2000 dilution of goat anti-PAT (*bar*) antibody (lot G863803) in 5% NFDM in PBST for 1 h at room temperature. Excess antibody was removed using three 10 min washes with PBST. Finally, the membrane was probed with horseradish peroxidase (HRP)-conjugated horse anti-goat IgG (Thermo, Rockford, IL) at a dilution of 1:10,000 in 5% NFDM in PBST for 1 h at room temperature. Excess HRP-conjugate was removed using three 10 min washes with PBST. All washes were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (GE, Healthcare, Piscataway, NJ) with exposure to Amersham Hyperfilm ECL (GE, Healthcare, Piscataway, NJ). The film was developed using a Konica SRX-101A automated film processor (Konica Minolta Medical & Graphic, Inc., Tokyo, Japan).

Quantification of the bands on 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 selection and contour tool. The signal intensities of the immunoreactive bands observed for the MON 88701- and *E. coli*-produced proteins migrating at the expected position on the blot film were quantified as "contour quantity" values. The raw data was exported to a Microsoft Excel (2007) file. The immunoreactivity of the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were reported as the mean signal intensity at each amount of protein analyzed. The immunoreactivity of the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were considered equivalent if the overall mean of the immunoreactive signal of the MON 88701-produced protein was within ± 35% of the overall mean of immunoreactive signal of the *E. coli*-produced PAT (*bar*) protein.

C.4.7.2. Results of PAT (*bar*) Protein Immunoreactivity Equivalence

Western blot analysis was conducted using goat anti- PAT (*bar*) polyclonal antibodies to 1) assess the identity of the PAT (*bar*) protein isolated from the cottonseed of MON 88701 and 2) to determine the relative immunoreactivity of the MON 88701- and the *E. coli*-produced PAT (*bar*) proteins. The results demonstrated that the anti-PAT (*bar*) antibodies recognized the MON 88701-produced PAT (*bar*) protein that migrated to an identical position on the blot as the *E. coli*-produced PAT (*bar*) protein (Figure C-11). Furthermore, the immunoreactive signal increased with increasing amounts of PAT (*bar*) protein loaded.

Densitometric analysis was conducted to compare the immunoreactivity of MON 88701- and the *E. coli*-produced PAT (*bar*) proteins. The mean signal intensity ($OD \times mm^2$) from the MON 88701-produced PAT (*bar*) bands and from the *E. coli*-produced PAT (*bar*) bands at each amount of protein analyzed was calculated and then overall mean signal intensity was calculated (Table C-12). The immunoreactivity was considered equivalent if the overall mean signal intensity of all MON 88701-produced PAT (*bar*) protein bands was within ±35% of the overall mean signal intensity of all *E. coli*-produced PAT (*bar*) protein bands.

The overall mean signal intensity of the *E. coli*-produced PAT (*bar*) bands was 4.669 $OD \times mm^2$, and the overall mean signal intensity of the MON 88701-produced PAT (*bar*) bands was 4.167 $OD \times mm^2$. Because overall mean signal intensity of the MON 88701-produced PAT (*bar*) protein bands was between 3.035 and 6.303 $OD \times mm^2$ (between -35% and +35% of the *E. coli*-produced PAT (*bar*) bands), the MON 88701-produced and *E. coli*-produced PAT (*bar*) proteins were determined to have equivalent immunoreactivity.

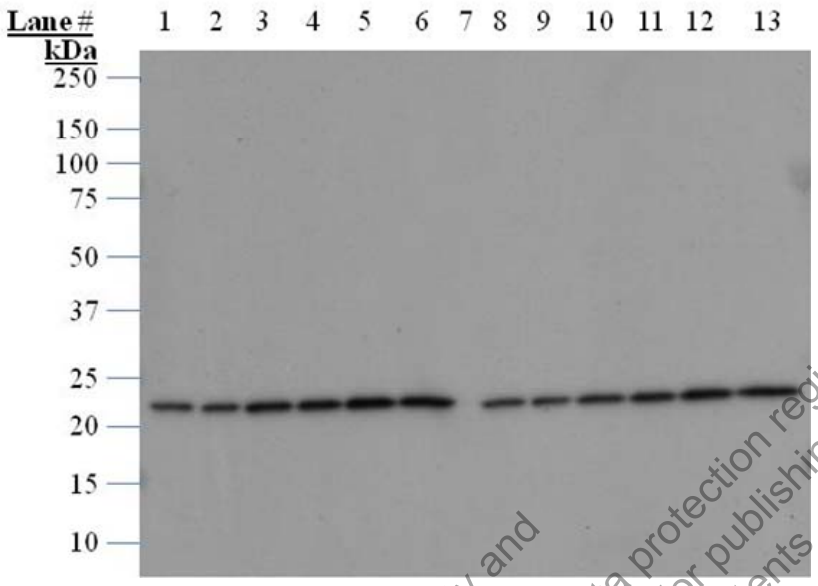

Figure C-11. Western Blot Analysis of the MON 88701- and *E. coli* -produced PAT (*bar*) Proteins

Aliquots of the MON 88701-produced PAT (*bar*) protein and the *E. coli*-produced PAT (*bar*) protein were subjected to SDS-PAGE and electrotransferred to a PVDF membrane. The membrane was incubated with anti-PAT (*bar*) antibodies and immunoreactive bands were visualized using an ECL system (GE Healthcare, Piscataway, NJ). Approximate molecular weights (kDa) are shown on the left. Lanes loaded with molecular weight markers were cropped, and lanes were renumbered relative to the original gel loading. The 1 min exposure is shown. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	*E. coli*-produced PAT (*bar*) protein	2
2	*E. coli*-produced PAT (*bar*) protein	2
3	*E. coli*-produced PAT (*bar*) protein	4
4	*E. coli*-produced PAT (*bar*) protein	4
5	*E. coli*-produced PAT (*bar*) protein	6
6	*E. coli*-produced PAT (*bar*) protein	6
7	Empty	-
8	MON 88701-produced PAT (*bar*) protein	2
9	MON 88701-produced PAT (*bar*) protein	2
10	MON 88701-produced PAT (*bar*) protein	4
11	MON 88701-produced PAT (*bar*) protein	4
12	MON 88701-produced PAT (*bar*) protein	6
13	MON 88701-produced PAT (*bar*) protein	6

Table C-12. Comparison of Immunoreactive Signals Between MON 88701- and *E. coli*-produced PAT (*bar*) Proteins

Mean Signal intensity from MON 88701-produced PAT (*bar*) (OD × mm^2)	Mean Signal intensity from *E. coli*-produced PAT (*bar*) (OD × mm^2)	Preset Acceptance limits for MON 88701-produced PAT (*bar*)[1] (OD × mm^2)
4.167	4.669	3.035 – 6.303

[1]The acceptance limits for the MON 88701-produced PAT (*bar*) are based on the interval between +35% (4.669 × 1.35) and -35% (4.669 × 0.65) of the overall mean of the *E. coli*-produced PAT (*bar*) signal intensity across all loads.

C.4.8. Molecular Weight and Purity Estimation using SDS-PAGE

C.4.8.1. Methods

MON 88701- and *E. coli*-produced PAT (*bar*) proteins were mixed with 5X LB and diluted with water to a final total protein concentration of 0.136 µg/µl. Molecular Weight Standards, Bio-Rad broad range (Hercules, CA) were diluted to a final total protein concentration of 0.9 µg/µl. The MON 88701-produced PAT (*bar*) was analyzed in duplicate at 1, 2, and 3 µg protein per lane. The *E. coli*-produced PAT (*bar*) reference standard was analyzed at 1 µg total protein in a single lane. The samples were loaded onto a 10-well pre-cast Tris glycine 4-20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA) and electrophoresis was performed at a constant voltage of 150 V for 95 min. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) acetic acid for 30 min, stained for 16.25 h with Brilliant Blue G-Colloidal stain (Sigma-Aldrich, St. Louis, MO). Gels were destained once for 30 to 45 sec with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, and four times for 2 h each (for a total of 8 h) with 25% (v/v) methanol. Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). The apparent MW of each observed band was estimated from a standard curve generated by the Quantity One software which was based on the MWs of the markers and their migration distance on the gel. To determine purity, all visible bands within each lane were quantified using Quantity One software. Apparent MW and purity were reported as an average of all six lanes containing the MON 88701-produced PAT (*bar*).

C.4.8.2. Results of PAT (*bar*) Protein Molecular Weight Equivalence

The molecular weight and purity of the PAT (*bar*) protein was determined to be 24.1 kDa and 99%, respectively. To assess apparent molecular weight (MW) and purity, the MON 88701-produced PAT (*bar*) protein was subjected to SDS-PAGE. The gel was stained with Brilliant Blue G Colloidal stain and analyzed by densitometry (Figure C-12). *E. coli*-produced PAT (*bar*) protein was loaded in a single lane for reference (Figure C-12, Lane 2). The MON 88701-produced PAT (*bar*) protein (Figure C-12, Lanes 3-8) had an apparent MW of 24.1 kDa (Table C-13). The apparent MW of the *E. coli*-produced PAT (*bar*) protein as reported on its Certificate of Analysis was 25.0 kDa (Table C-8). Because the apparent MW of MON 88701-produced PAT (*bar*) protein was within the preset acceptance limits (Table C-13), the MON 88701-produced and *E. coli*-produced PAT (*bar*) proteins were determined to have equivalent apparent MWs.

The purity of the MON 88701-produced PAT (*bar*) protein was calculated based on the six loads on the gel (Figure C-12, Lanes 3-8). The average purity was determined to be more than 99%.

Table C-13. Molecular Weight Comparison Between the MON 88701- and *E. coli*-produced PAT (*bar*) Proteins Based on SDS-PAGE

Apparent Molecular Weight of MON 88701-Produced PAT (*bar*) Protein[1] (kDa)	Apparent Molecular Weight of *E. coli*-Produced PAT (*bar*) Protein[2] (kDa)	Preset Acceptance Limits for MON 88701-produced PAT (*bar*)[3] (kDa)
24.1	25.0	23.9-25.4

[1]The reported value is the mean molecular weight across all six loads.
[2]The molecular weight of the *E. coli*-produced PAT (*bar*) protein as reported on its Certificate of Analysis.
[3]See Section C.6.

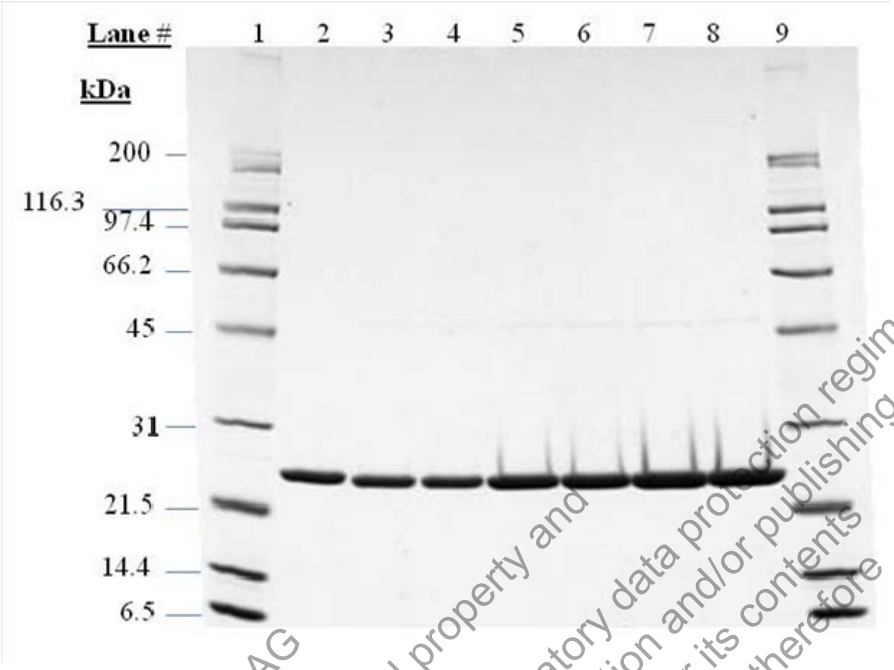

Figure C-12. Molecular Weight and Purity Analysis of the MON 88701-produced PAT (*bar*) Protein

Aliquots of the MON 88701-produced and the *E. coli*-produced PAT (*bar*) proteins were subjected to SDS-PAGE and then stained with Brilliant Blue G-Colloidal stain. Approximate molecular weights are shown on the left and correspond to the markers loaded in Lanes 1 and 9. Empty lane was partially cropped. Lane designations are as follows:

Lane	Sample	Amount (µg)
1	Broad Range Molecular Weight Markers	4.5
2	*E. coli*-produced PAT (*bar*) protein	1
3	MON 88701-produced PAT (*bar*) protein	1
4	MON 88701-produced PAT (*bar*) protein	1
5	MON 88701-produced PAT (*bar*) protein	2
6	MON 88701-produced PAT (*bar*) protein	2
7	MON 88701-produced PAT (*bar*) protein	3
8	MON 88701-produced PAT (*bar*) protein	3
9	Broad Range Molecular Weight markers	4.5

C.4.9. Glycosylation Analysis

C.4.9.1. Methods

Glycosylation analysis was used to determine whether the MON 88701-produced PAT (*bar*) was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 88701-produced PAT (*bar*) protein, the *E. coli*-produced PAT (*bar*) (negative control) and the positive control, transferrin (Sigma-Aldrich, St. Louis, MO), were each diluted with water and brought to 1X LB. These samples were heated at ~102 °C for 4 min. The MON 88701-produced PAT (*bar*), the *E. coli*-produced PAT (*bar*) and transferrin were loaded at approximately 50 and 100 ng per lane on a Tris-glycine 10 well 4-20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA). Precision Plus Protein Dual color Standards (Bio-Rad, Hercules, CA) were also loaded to verify electrotransfer of the proteins to the membrane and as markers for molecular weight. Electrophoresis was performed at a constant voltage of 150 V for 90 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 60 min at a constant current of 200 mA.

Carbohydrate detection was performed directly on the PVDF membrane at room temperature using the Amersham ECL glycoprotein Detection Module (GE, Healthcare, Piscataway, NJ). With this module, carbohydrate moieties of proteins were oxidized with sodium metaperiodate and then biotinylated with biotin-X-hydrazide. The biotinylated proteins can be detected on the blot by addition of streptavidin conjugated to HRP for luminol-based detection using ECL reagents (GE, Healthcare, Piscataway, NJ) and with subsequent exposure to Amersham Hyperfilm (GE, Healthcare). The film was developed using a Konica SRX-101A automated film processor (Konica Minolta Medical & Graphic, Inc., Tokyo, Japan).

An identical blot run in parallel to that used for the glycosylation analysis was stained to visualize the proteins present on the membrane. Proteins were stained for 30 sec to 2 min using Coomassie Brilliant Blue R-250 staining solution (Bio-Rad, Hercules, CA) and then destained with 1X Coomassie Brilliant Blue R-250 destaining solution (Bio-Rad) for more than 5 min. After washing with water, the blot was dried and scanned using Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0).

C.4.9.2. Results of Glycosylation Analysis

Some eukaryotic proteins are post-translationally modified by the addition of carbohydrate moieties (Rademacher et al., 1988). To test whether PAT (*bar*) protein was glycosylated when expressed in the cottonseed of MON 88701, the MON 88701-produced PAT (*bar*) protein was analyzed using an ECL Glycoprotein Detection Module (GE, Healthcare, Piscataway, NJ). Transferrin, a glycosylated protein, was used as a positive control in the assay. To assess equivalence of the MON 88701- and *E. coli*-produced PAT (*bar*) proteins, the *E. coli*-produced PAT (*bar*) protein was also analyzed. The positive control was clearly detected at the expected molecular weight (~80 kDa) and the band intensity increased with increasing concentration (Figure C-13, Panel A, Lanes 1-2). In contrast, signals were not observed in the lanes

containing the MON 88701- or *E. coli*-produced protein at the expected molecular weight for the PAT (*bar*) protein (Figure C-13 Panel A, Lanes 7-8 and Lanes 4-5, respectively). To assess whether the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were loaded appropriately for glycosylation analysis, a second membrane (with identical loadings and transfer times) was stained with Coomassie Blue R-250 for protein detection (Figure C-13 Panel B). Both the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were clearly detected (Figure C-13 Panel B, Lanes 7-8 and Lanes 4-5, respectively). These data indicate that the glycosylation status of MON 88701-produced PAT (*bar*) protein and *E. coli*-produced PAT (*bar*) protein are equivalent and that neither is glycosylated.

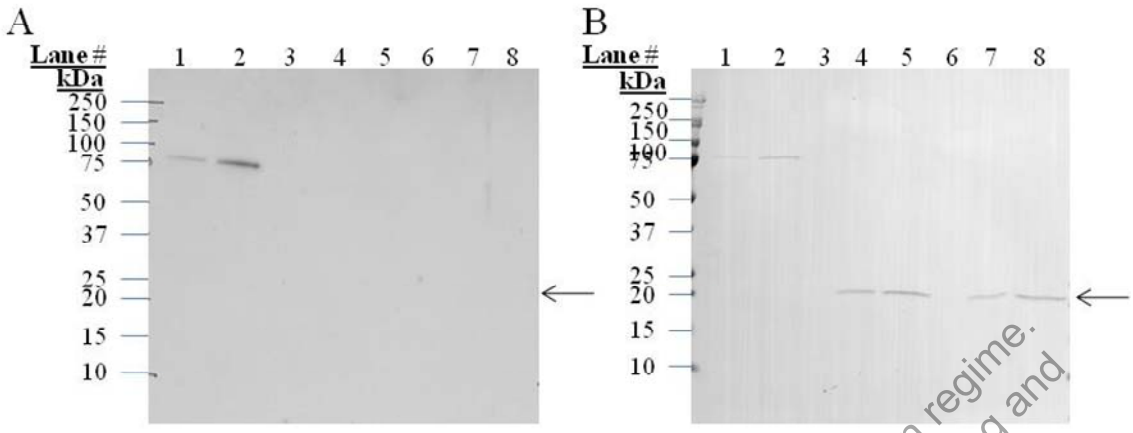

Figure C-13. Glycosylation Analysis of the MON 88701-produced PAT (*bar*) Protein

Aliquots of the transferrin (positive control), *E. coli*-produced PAT (*bar*) protein and MON 88701-produced PAT (*bar*) protein were subjected to SDS-PAGE and electrotransferred to PVDF membranes. Panel A corresponds to detection of labeled carbohydrate moieties, where present, using the ECL-based system with exposure to Hyperfilm. A 7 min exposure is shown. Panel B corresponds to Coomassie Blue R-250 staining of an equivalent blot to confirm the presence of proteins. The signal was captured using a Bio-Rad GS-800 with Quantity One software (version 4.4.0). Approximate molecular weights (kDa) correspond to the Precision Plus, dual color markers (used to verify transfer and MW). Lanes loaded with molecular weight markers were cropped, and lanes were renumbered relative to the original gel loading. Arrows indicate the expected migration of PAT (*bar*) protein. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	Transferrin (positive control)	50
2	Transferrin (positive control)	100
3	Empty	-
4	*E. coli*-produced PAT (*bar*) (negative control)	50
5	*E. coli*-produced PAT (*bar*) (negative control)	100
6	Empty	-
7	MON 88701-produced PAT (*bar*)	50
8	MON 88701-produced PAT (*bar*)	100

C.4.10. Functional Activity Analysis

C.4.10.1. Methods

PAT (*bar*) catalyzes the reaction of phosphinothricin (PPT) with acetyl CoA to form acetyl PPT and free CoA. To assess functional activity of PAT (*bar*), the amount of CoA released during the reaction can be monitored using the reduction of 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) by CoA to form the colorimetric reagent 5-thio-nitrobenzoate (TNB) (Wehrmann et al., 1996).

Prior to functional activity analysis, both MON 88701- and *E. coli*-produced PAT (*bar*) proteins were diluted to a purity corrected concentration of 1 ng/µl with a 50 mM Tris, pH 7.5, and 0.5 mM EDTA buffer. Assays for both proteins were conducted using five replicates. The reaction mixtures containing 2 mM acetyl CoA, 1 mM DTNB, 50 mM Tris, pH 7.8, and 0.5 mM EDTA with or without 1 mM phosphinothricin were pre incubated at ~30 °C for 10-60 min. The reactions were then initiated by the addition of 10 ng of PAT (*bar*) enzyme. The reaction rate was monitored in each well at 412 nm and ~30 °C using a plate reader in one minute intervals for 30 min. A response curve was prepared using 3.9 µM to 250 µM β-mercaptoethanol in 1 mM DTNB, 50 mM Tris, pH 7.8, and 0.5 mM EDTA. The response curve was generated only to verify assay conditions and instrument performance. The initial assay results are reported as the mean velocity of the reaction of PAT (*bar*) (generated by the KC4 software, Power Wave Xi, Bio Tek, Richmond, VA) and expressed as min^{-1}. The specific activities of the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were then calculated using the molar absorptivity of product released during the assay, TNB (13,600 $M^{-1} \times cm^{-1}$ or 13.6 $\mu mol^{-1} \times ml$). Specific activity is expressed as µmol of TNB released per minute per mg of PAT (*bar*) ($\mu mol \times min^{-1} \times mg^{-1}$). Calculations of the specific activities were performed using Microsoft Excel (2007).

C.4.10.2. Results of Functional Activity

The functional activities of the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were assessed using a colorimetric assay that measures PAT (*bar*) catalyzed release of coenzyme A (CoA) from acetyl-CoA upon transfer of an acetyl-group to phosphinothricin. In this assay, protein-specific activity is expressed as $\mu mol \times minute^{-1} \times mg^{-1}$ of PAT enzyme.

The experimentally determined specific activities for the MON 88701- and *E. coli*-produced PAT (*bar*) proteins are presented in Table C-14. The specific activities of MON 88701- and *E. coli*-produced PAT (*bar*) proteins were 36.4 and 46.2 $\mu mol \times minute^{-1} \times mg^{-1}$ of PAT (*bar*), respectively. Because the specific activities of the MON 88701-produced and *E. coli*-produced PAT (*bar*) proteins fall within the preset acceptance criterion (Table C-14), the MON 88701- and *E. coli*-produced PAT (*bar*) proteins were determined to have equivalent functional activity.

Table C-14. PAT (*bar*) Functional Activity

MON 88701-produced PAT (*bar*)[1] (µmol × minute^{-1} × mg^{-1})	*E. coli*-produced PAT (*bar*)[1] (µmol × minute^{-1} × mg^{-1})	Preset Acceptance Limits for MON 88701-produced PAT (*bar*)[2] (µmol × minute^{-1} × mg^{-1})
36.4 ± 1.3	46.2 ± 2.1	30.17 - 51.70

[1]Value refers to mean and standard deviation calculated based on n = 5.
[2]See Section C.6.

C.5. Substrate Specificity of PAT (*bar*) Protein Produced in MON 88701

The PAT proteins, including PAT (*bar*) protein, were demonstrated to be highly specific for glufosinate in the presence of acetyl-CoA. Since the specificity of PAT proteins has been well established in literature, and due to the lack of any documented reports of non-specific effects of PAT proteins since the introduction of glufosinate tolerant crops in 1995, in-house experiments were not conducted to further demonstrate substrate specificity of PAT (*bar*) protein isolated from MON 88701.

C.6. Prediction Intervals as Acceptance criteria

Acceptance criteria (acceptance limits) based on prediction intervals were used to assess the equivalence of the MON 88701-produced and *E. coli*-produced proteins for apparent MW and functional activity. A prediction interval is an estimate of an interval in which a randomly selected future observation from a population will fall, with a certain degree of confidence, given what has already been observed (Hahn and Meeker, 1991a; b); *i.e.*, prediction intervals are generated based on the statistical analysis of the existing data. Data obtained from multiple assays of *E. coli*-produced protein conducted under GLP guidelines were used for this purpose.

To generate the 95% prediction interval (PI), the mean and standard deviation of the data from several assays were calculated. The number of assays used to calculate the mean and the number of future assays (one for equivalence studies) were used in the following formula to generate the PI:

$$\overline{\mathrm{X}} \pm r(1-\alpha;\ m,n) \quad (s)$$

$r_{(1-\alpha;\ m,\ n)}$ is estimated using the formula given below:

$$r_{(1-\alpha;m,n)} \cong t_{(1-.05/(2m);\ n-1)}\sqrt{1+\frac{1}{n}}$$

Where $\bar{X}$ is mean of the replicate assays; s is standard deviation of the replicates; $1-\alpha$ is the level of confidence; n is the number of assays used to generate the mean; and m is the number of future assays (one for equivalence studies). The t-value is the $100(1-.05/(2m))^{th}$ percentile from Student's t-distribution with n-1 degrees of freedom. With 95% confidence, all m future values of the assay will fall within this interval (Hahn and Meeker, 1991a; b). If the assay means do not appear to have been derived from a normal distribution, but the logarithms of the raw values do follow a normal distribution, then prediction intervals may be applied to the logarithms of the raw values (Hahn and Meeker, 1991a; b).

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Appendix D: Materials and Methods Used for the Analysis of the Levels of MON 88701 DMO and PAT (*bar*) Proteins in MON 88701

D.1. Materials

Seed, over season leaf (OSL-1-4), root, and pollen tissue samples from dicamba and glufosinate-treated MON 88701 were harvested from eight field sites in the U.S. during the 2010 growing season from starting seed lot 11268129, with the exception of OSL-1 (7 sites) and OSL-4 (7 sites). MON 88701 plots were treated at the 3-5 leaf stage with glufosinate herbicide at the label rate (0.5 lbs a.i./acre) and at the 6-10 leaf stage with dicamba herbicide at the label rate (0.5 lbs a.e./acre). *E. coli*-produced MON 88701 DMO (lot 11293429) and PAT (*bar*) protein (lot 11270310) were used as the analytical reference standards.

D.2. Characterization of the Materials

The identity of MON 88701 was confirmed by conducting MON 88701 event specific polymerase chain reaction (PCR) analyses on the harvested seed from each site. Any seed sample and its associated tissues for which three or more pools out of four tested unexpectedly during PCR verification were not analyzed in this study.

D.3. Field Design and Tissue Collection

Field trials were initiated during the 2010 planting season to generate MON 88701 seed, OSL-1-4, root, and pollen samples at various cotton growing locations in the U.S. The tissue samples from the following field sites were analyzed: Arkansas (ARTI), Georgia (GACH), Kansas (KSLA), Louisiana (LACH), North Carolina (NCBD), New Mexico (NMLC), South Carolina (SCEK) and Texas (TXPL). These field sites were representative of cotton producing regions suitable for commercial production. At each site, four replicated plots of plants containing MON 88701 were planted using a randomized complete-block field design. Seed, over season leaf (OSL-1-4), root, and pollen samples were collected from each replicated plot at all field sites, except OSL-1 at site TXPL and OSL-4 at site LACH. See Tables V-4 and V-5 for detailed descriptions of when the samples were collected.

D.4. Tissue Processing and Protein Extraction

Tissue samples were shipped to Monsanto Company (St. Louis, Missouri), and were prepared by the Monsanto Sample Management Team. The prepared tissue samples were stored in a -80° C freezer until transferred on dry ice to the analytical facility.

D.4.1. MON 88701 DMO Protein

MON 88701 DMO protein was extracted from tissue samples as described in Table D-1. MON 88701 DMO was extracted from over season leaf (OSL-1-4) and root tissues samples with the appropriate amount of Tris borate buffer with 0.5% (w/v) bovine serum albumin (1 × TB + 0.5% BSA) [0.1 M Tris, 0.1 M $Na_2B_4O_7$, 0.05 M $MgCl_2$, 0.05% (v/v)

Tween 20 at pH 7.8, 0.5% (w/v) BSA]. MON 88701 DMO was extracted from pollen and seed tissues with the appropriate amount of phosphate buffered saline (PBS) with Tween 20 (1 × PBST). Extractions were done using 8 1/4″ chrome-steel beads, and shaking in a Harbil mixer (Fluid Management, Wheeling, Illinois). Insoluble material was removed from all tissue extracts using a serum filter (Fisher Scientific, Pittsburgh, PA) The extracts were aliquoted and stored frozen in a -80 °C freezer until ELISA analysis.

Table D-1. MON 88701 DMO Protein Extraction Methods for Tissue Samples

Sample Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf[1]	1:100	1 × TB + 0.5% BSA
Root	1:100	1 × TB + 0.5% BSA
Pollen	1:100	1 × PBST
Seed	1:100	1 × PBST

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

D.4.2. PAT (*bar*) Protein

PAT (*bar*) protein was extracted from tissue samples as described in Table D-2. PAT (*bar*) was extracted from over season leaf (OSL-1-4) and root tissues samples with the appropriate amount of Tris borate buffer with L-ascorbic acid (1× TBA) [0.1 M Tris, 0.1 M $Na_2B_4O_7$, 0.05 M $MgCl_2$, 0.05% (v/v) Tween 20 at pH 7.8, 0.2% (w/v) L-ascorbic acid]. PAT (*bar*) was extracted from pollen and seed tissues with the appropriate amount of 1 × PBST. Extractions were done using 8 1/4″ chrome-steel beads, and shaking in a Harbil mixer. Insoluble material was removed from all tissue extracts using a serum filter. The extracts were aliquoted and stored frozen in a -80 °C freezer until ELISA analysis.

Table D-2. PAT (*bar*) Protein Extraction Methods for Tissue Samples

Sample Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf[1]	1:100	1× TBA
Root	1:100	1× TBA
Pollen	1:100	1× PBST
Seed	1:100	1× PBST

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

D.5. Protein Antibodies

D.5.1. MON 88701 DMO Protein

Goat polyclonal antibodies specific for the DMO protein were purified using Protein G affinity chromatography. The concentration of the purified IgG was determined to be 8.1 mg/ml by spectrophotometric methods. The purified antibody was stored in 1× PBS.

Protein G-affinity purified goat polyclonal anti-DMO antibodies were coupled with biotin (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instructions. The detection reagent was NeutrAvidin (Thermo Fisher Scientific, Rockford, IL) conjugated to horseradish peroxidase (HRP).

D.5.2. PAT (*bar*) Protein

Goat polyclonal PAT (*bar*)-specific IgG was purified by Protein G-affinity chromatography followed by PAT (*bar*) antigen affinity chromatography. The concentration of the purified IgG was determined to be 3.6 mg/ml by spectrophotometric methods. The purified antibody was stored in 1× PBS.

Protein G-affinity purified goat polyclonal anti-PAT (*bar*) antibodies were coupled with biotin (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instructions. The detection reagent was NeutrAvidin (Thermo Fisher Scientific, Rockford, IL) conjugated to horseradish peroxidase (HRP).

D.6. Protein ELISA Method

D.6.1. MON 88701 DMO Protein

Goat anti-DMO antibodies were diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM NaCl) to a final concentration of 5 µg/ml and immobilized onto 96 well microtiter plates followed by incubation in a 4° C refrigerator for > 8 hours. Prior to each step in the assay, plates were washed with 1× PBST. Plates were blocked with the addition of 200 µl per well of blocking buffer, Blocker Casein (Thermo Fisher Scientific, Rockford, IL) in Tris Buffered Saline (TBS) for 60 to 70 minutes at room temperature (RT). DMO protein standard or sample extract was added at 100 µl per well and incubated for 60 to 65 minutes at 37° C. Biotinylated goat anti-DMO antibodies prepared in 1× Tris-borate buffer with 10% Blocker Casein in TBS were added at 100 µl per well and incubated for 60 to 65 minutes at 37° C. NeutrAvidin HRP conjugate was added at 100 µl per well and incubated for 30 to 35 minutes at 37° C. Plates were developed by adding 100 µl per well of substrate, 3,3',5,5' tetramethyl benzidine (TMB; Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 3 M H_3PO_4. Quantification of the DMO protein was accomplished by interpolation from a DMO protein standard curve that ranged from 0.313 – 10 ng/ml.

D.6.2. PAT (*bar*) Protein

Affinity purified goat anti PAT (*bar*) antibodies were diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM sodium chloride) to a final concentration of 4 µg/ml and immobilized onto 96 well microtiter plates, followed by incubation in a 4° C refrigerator for >12 h. Prior to each step in the assay, plates were washed with 1× PBST. Plates were blocked with the addition of 200 µl per well of blocking buffer (1× PBST+1% BSA) for 60 to 70 minutes at 37° C. PAT (*bar*) protein standard or sample extract was added at 100 µl per well and incubated for 60 to 70 minutes at 37° C. Biotinylated goat anti-PAT (*bar*) antibodies diluted in 1 × PBST + 0.1% BSA were added at 100 µl per well and incubated for 60 to 70 minutes at 37° C. NeutrAvidin HRP conjugate was added at 100 µl per well and incubated for 60 to 70 minutes at 37° C. Plates were developed by adding 100 µl per well of TMB substrate. The enzymatic reaction was terminated by the addition of 100 µl per well of 3 M H_3PO_4. Quantification of the PAT (*bar*) protein was accomplished by interpolation from a PAT (*bar*) protein standard curve that ranged from 0.625 – 20 ng/ml.

D.7. Moisture Analysis

Tissue moisture content was determined using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). A homogeneous tissue-specific site pool (TSSP) was prepared consisting of samples of a given tissue type grown at a given site. The average percent moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$\text{DWCF} = 1 - \left(\frac{\text{Mean\% TSSP Moisture}}{100}\right)$$

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

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

Due to a limited amount of tissue, pollen was not analyzed for moisture content. Therefore, no dry weight calculation was performed and pollen was reported on a µg/g fresh weight (fw) basis only.

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

D.8. Data Analyses

All MON 88701 DMO and PAT (*bar*) ELISA plates were analyzed on a SPECTRAmax Plus 384 (Molecular Devices, Sunnyvale, CA) microplate spectrophotometer, using a dual wavelength detection method. All protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of

620-650 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GxP version 5.0.1 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 fw basis for data that were greater than or equal to the LOQ. This conversion utilized a sample dilution factor, and tissue-to-buffer ratio. The protein values in µg/g fw were also converted to µg/g dw by applying the DWCF (except pollen). Microsoft Excel 2007 (Microsoft, Redmond, WA) was used to calculate the protein levels in all cotton tissues. The sample means, standard deviations, and ranges were also calculated by Microsoft Excel 2007. All protein expression levels were rounded to two significant figures.

Any MON 88701 sample extracts that resulted in an unexpectedly negative result by ELISA analysis was re extracted twice for the protein of interest and re analyzed by ELISA to confirm the results. Samples with confirmed unexpected results were omitted from all calculations.

Appendix E: Materials, Methods, and Individual Site Results for Compositional Analysis of MON 88701 Cottonseed

E.1. Materials

Cottonseed from MON 88701 (Seed Lot Number 11268129) treated with dicamba and glufosinate (T) and MON 88701 not treated with dicamba or glufosinate (NT) and the conventional control (Seed Lot Number 11268128) was evaluated. The conventional control has background genetics similar to that of MON 88701 but does not contain either the dicamba mono-oxygenase (DMO) or phosphinothricin N-acetyltransferase (PAT) proteins. The commercial reference varieties were nine conventional cotton varieties (Table E-1).

Table E-1. Commercial Reference Varieties

Material Name	Seed Lot No.	Field Sites[1]
SG 125	11266155	ARTI, SCEK, NCBD, TXPL
DP 435	11266762	ARTI, NCBD, TXPL
DP 5415	11266157	ARTI, LACH, KSLA
FM 989	10001810	ARTI, LACH, GACH
Delta Opal	11266158	SCEK, GACH, NCBD
Atlas	11266765	SCEK, TXPL, KSLA, NMLC
ST 474	11266156	GACH, LACH, NCBD, NMLC, SCEK
DP 565	11266764	GACH, LACH, KSLA, NMLC
NM 1517-99	11268233	TXPL, KSLA

[1]Field sites described in Section VII.A.

E.2. Characterization of the Materials

The identities of MON 88701(T) and MON 88701(NT), the conventional control, and commercial reference varieties were confirmed by verifying the chain of custody documentation prior to analysis. To further confirm the identities of MON 88701(T) and MON 88701(NT), the conventional control, and commercial reference varieties, event-specific polymerase chain reaction (PCR) analyses were conducted on the harvested, acid-delinted cottonseed from each site to confirm the presence or absence of the MON 88701 event.

E.3. Field Production of the Samples

Cottonseed samples were collected from MON 88701(T) and MON 88701(NT) and the conventional control Coker 130 grown in a 2010 U.S. field production. Four different conventional cotton varieties, known as reference substances, were included at each site of the field production to provide data on natural variability of each compositional

component analyzed. The field production was conducted at eight sites: Arkansas (ARTI), Georgia (GACH), Kansas (KSLA), Louisiana (LACH), North Carolina (NCBD), New Mexico (NMLC), South Carolina (SCEK) and Texas (TXPL). The sites were planted in a randomized complete block design with four blocks per site. MON 88701 plots were treated at the 3-5 leaf stage with glufosinate herbicide at the label rate (0.5 lbs a.i. /acre) and at the 6-10 leaf stage with dicamba herbicide at the label rate (0.5 lbs a.e./acre). T/C/R substances were grown under normal agronomic field conditions for their respective geographic regions. Cottonseed samples were harvested and ginned from all plots and shipped at ambient temperature to Monsanto Company (St. Louis, Missouri). The samples were acid-delinted and a subsample was obtained from each for compositional analyses. These subsamples were ground and stored in a freezer set to maintain –20°C until their shipment on dry ice to Covance Laboratories Inc. (Madison, Wisconsin) for analysis. The label on the samples shipped listed the protocol (study) number, tissue type, material name, storage conditions, and a unique sample ID number.

E.4. Summary of Analytical Methods

Harvested, acid-delinted cottonseed samples were analyzed by Covance Laboratories Inc. Upon receipt, the samples were stored in a freezer set to maintain -20 °C until their use. Nutrients assessed in this analysis included proximates (ash, fat, moisture, protein, and carbohydrates and calories by calculation), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber (CF), total dietary fiber (TDF), amino acids (AA), fatty acids (C8-C22), minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn) and vitamin E (α-tocopherol). Anti-nutrients analyzed included gossypol and cyclopropenoid fatty acids (CPFA).

E.4.1. Acid Detergent Fiber

The ANKOM2000 Fiber Analyzer automated the process of removal of proteins, carbohydrates, and ash. Fats and pigments were removed with an acetone wash prior to analysis. The fibrous residue that is primarily cellulose, lignin, and insoluble protein complexes remained in the Ankom filter bag, and were determined gravimetrically. (Komarek, et al., 1993; USDA, 1970). The results were reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.2. Amino Acid Composition

The following 18 amino acids were analyzed:

Total threonine	Total aspartic acid (including asparagine)
Total serine	Total tyrosine
Total phenylalanine	Total glutamic acid (including glutamine)
Total proline	Total histidine
Total glycine	Total lysine
Total alanine	Total arginine
Total valine	Total tryptophan
Total isoleucine	Total methionine
Total leucine	Total cystine (including cysteine)

The samples were hydrolyzed in 6N hydrochloric acid for approximately 24 hours at approximately 106-110ºC. Phenol was added to the 6N hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine are converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid. Tryptophan was hydrolyzed from proteins by heating at approximately 110ºC in 4.2N sodium hydroxide for 20 hours. The samples were analyzed by HPLC after pre-injection derivatization. The primary amino acids were derivatized with o-phthalaldehyde (OPA) and the secondary amino acids are derivatized with fluorenylmethyl chloroformate (FMOC) before injection. (AOAC, 2011a; Barkholt and Jensen, 1989; [redacted] and Brooks, 2010; [redacted], et al., 2000; Schuster, 1988). The results were reported on fresh weight basis. The limit of quantitation was 0.100 mg/g.

Reference Standards:

Component	Manufacturer	Lot No.	Purity (%)
L-Alanine	Sigma-Aldrich	1440397	99.9
L-Arginine Monohydrochloride	Sigma-Aldrich	1361811	100
L-Aspartic Acid	Sigma-Aldrich	BCBB9274	100.6
L-Cystine	Sigma-Aldrich	1418036	99.9
L-Glutamic Acid	Sigma-Aldrich	1423805	100.2
Glycine	Sigma-Aldrich	1119375	100
L-Histidine Monohydrochloride Monohydrate	Sigma-Aldrich	BCBB1348	99.9
L-Isoleucine	Sigma-Aldrich	1423806	100
L-Leucine	Sigma-Aldrich	BCBB1733	98.6
L-Lysine Monohydrochloride	Sigma-Aldrich	1362380	100.2
L-Methionine	Sigma-Aldrich	1423807	99.9
L-Phenylalanine	Sigma-Aldrich	BCBB9200	100
L-Proline	Sigma-Aldrich	1414414	99.7
L-Serine	Sigma-Aldrich	1336081	99.9
L-Threonine	Sigma-Aldrich	1402329	100
L-Tryptophan	Sigma-Aldrich	BCBB1284	99.8
L-Tyrosine	Sigma-Aldrich	BCBB5393	99.5
L-Valine	Sigma-Aldrich	1352709	100

E.4.3. Ash

The sample was placed in an electric furnace at 550 °C and ignited. The nonvolatile matter remaining was quantified gravimetrically and calculated to determine percent ash (AOAC, 2011b). The limit of quantitation was 0.100%.

E.4.4. Calories

Calories were calculated using the Atwater factors with the fresh weight-derived data and the following equation:

$$\text{calories (Kcal/100g)} = (4 \times \%\ \text{protein}) + (9 \times \%\ \text{fat}) + (4 \times \%\ \text{carbohydrates})$$

The limit of quantitation was calculated as 2.00 Kcalories/100g on a fresh weight basis (Code of Federal Regulation, Title 21, Part 101.9, pp. 24-25).

E.4.5. Carbohydrates

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

$$\%\ \text{carbohydrates} = 100\% - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{moisture} + \%\ \text{ash})$$

The results were reported on fresh weight basis (USDA, 1973). The limit of quantitation was 0.100%.

E.4.6. Crude Fiber

Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions (AOAC, 2011c). The results were reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.7. Fat by Soxhlet Extraction

The sample was weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed (AOAC, 2011d; e). The results were reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.8. Cyclopropenoid Fatty Acids

The total lipid fraction was extracted from the sample using chloroform and methanol. A portion of the lipid fraction was then saponified with a mild alkaline hydrolysis. The free fatty acids were extracted with ethyl ether and hexane. The free fatty acids were then converted to their phenacyl derivatives with 2-bromoacetophenone. The derivatives were quantitated on a high-performance liquid chromatography system equipped with an ultraviolet detector. The amount of malvalic, sterculic and dihydrosterculic acids were determined by comparison to an external calibration curves of similarly derivatized reference standards (Wood, 1986). The results were expressed on a fresh weight basis. The limit of quantitation was 50.0 µg/g.

Reference Standards:

- Monsanto, Malvalic Acid, 100%, Lot Number GLP-0208-12964-A
- Monsanto, Sterculic Acid, 99%, Lot Number GLP-0208-12963-A
- Monsanto, Dihydrosterculic, 98%, Lot Number GLP-0311-14467-A

E.4.9. Fatty Acids

The lipid was extracted and saponified with 0.5 N methanolic sodium hydroxide, and methylated with 14% boron trifluoride in methanol. The resulting methyl esters of the fatty acids were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (AOCS, 1997; 2001; 2009a; c). The results were reported on fresh weight basis. The limit of quantitation was 0.0200%.

Reference Standards:

Component	Lot Number	Component	Weight (%)	Purity (%)
Nu-Chek Prep GLC Reference Standard Hazelton No. 1	JY20-U	Methyl Octanoate	16.66	99.6
		Methyl Decanoate	16.66	99.6
		Methyl Laurate	16.66	99.8
		Methyl Myristate	16.66	99.8
		Methyl Palmitoleate	16.66	99.7
		Methyl Linolenate	16.66	99.5
Nu-Chek Prep GLC Reference Standard Hazelton No. 2	AU16-U	Methyl Arachidate	33.33	99.6
		Methyl 11-Eicosenoate	33.33	99.5
		Methyl Arachidonate	33.33	99.6
Nu-Chek Prep GLC Reference Standard Hazelton No. 3	J28-U	Methyl Myristoleate	12.5	99.5
		Methyl Pentadecanoate	12.5	99.6
		Methyl 10-Pentadecenoate	12.5	99.5
		Methyl Heptadecanoate	12.5	99.6
		Methyl 10-Heptadecenoate	12.5	99.5
		Methyl 11-14 Eicosadienoate	12.5	99.6
		Methyl Behenate	12.5	99.8
		Methyl 11-14-17 Eicosatrienoate	12.5	99.5
Nu-Chek Prep GLC Reference Standard Hazelton No. 3	F15-V	Methyl Myristoleate	12.5	99.5
		Methyl Pentadecanoate	12.5	99.6
		Methyl 10-Pentadecenoate	12.5	99.5
		Methyl Heptadecanoate	12.5	99.6
		Methyl 10-Heptadecenoate	12.5	99.5
		Methyl 11-14 Eicosadienoate	12.5	99.6
		Methyl Behenate	12.5	99.8
		Methyl 11-14-17 Eicosatrienoate	12.5	99.5
Nu-Chek Prep GLC Reference Standard Hazelton No. 4	MA30-U	Methyl Palmitate	27.0	99.6
		Methyl Stearate	19.0	99.5
		Methyl Oleate	27.0	99.8
		Methyl Linoleate	27.0	99.8
Nu-Chek Prep GLC Reference Standard Hazelton No. 4	JA31-V	Methyl Palmitate	27.0	99.7
		Methyl Stearate	19.0	99.7
		Methyl Oleate	27.0	99.8
		Methyl Linoleate	27.0	99.8
Nu-Chek Prep Methyl Gamma Linolenate	U-63M-M18-U	Not applicable	Not applicable	>99
Nu-Chek Prep Methyl Gamma Linolenate	U-63M-N2-U	Not applicable	Not applicable	>99
Nu-Chek Prep Methyl Tridecanoate	N-13M-F16-V	Not applicable	Not applicable	>99
Nu-Chek Prep Methyl Tridecanoate	N-13M-MA25-T	Not applicable	Not applicable	>99

E.4.10. Free and Total Gossypol

For free gossypol, the sample was extracted with aqueous acetone. The solution was then filtered and the free gossypol was reacted with aniline. For total gossypol analysis, the sample was extracted using a complexing reagent containing acetic acid, 3-amino-1-propanol, and dimethylformamide. The solution was then filtered and the total gossypol was reacted with aniline. For both analyses, the dianilinogossypol was quantitated spectrophotometrically using a standard curve (AOCS, 2011a; b) The results were reported on fresh weight basis. The limit of quantitation was 0.00200%.

Reference Standard:

- Sigma-Aldrich, Gossypol, 97.7%, Lot Number 059K4046

E.4.11. ICP Emission Spectrometry

The sample was dried, precharred, and ashed overnight in a muffle furnace set to maintain 500 °C. The ashed sample was re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured on the inductively coupled plasma spectrometer, with the emission of the standard solutions (AOAC, 2011f; g). The results were reported on fresh weight basis.

Inorganic Ventures Reference Standards and Limits of Quantitation

Mineral	Lot Numbers	Concentration (µg/mL)	Limit of Quantitation (ppm)
Calcium	E2-MEB360079MCA, E2-MEB360081	200, 1000	20.0
Copper	E2-MEB360079MCA, E2-MEB360080MCA	2.00, 10.0	0.500
Iron	E2-MEB360079MCA, E2-MEB360082	10.0, 50.0	2.00
Magnesium	E2-MEB360079MCA, E2-MEB360080MCA	50.0, 250	20.0
Manganese	E2-MEB360079MCA, E2-MEB360080MCA	2.00, 10.0	0.300
Phosphorus	E2-MEB360079MCA, E2-MEB360081	200, 1000	20.0
Potassium	E2-MEB360079MCA, E2-MEB360081	200, 1000	100
Sodium	E2-MEB360079MCA, E2-MEB360081	200, 1000	100
Zinc	E2-MEB360079MCA, E2-MEB360080MCA	10.0, 50.0	0.400

E.4.12. Moisture

The sample was dried in a vacuum oven at approximately 100 °C to a constant weight. The moisture weight loss was determined and converted to percent moisture (AOAC, 2011h; i). The results were reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.13. Neutral Detergent Fiber, Enzyme Method

The ANKOM2000 Fiber Analyzer automated the process of the removal of proteins, carbohydrates, and ash. The fats and pigments were removed with an acetone wash prior to analysis. Hemicellulose, cellulose, lignin, and insoluble protein fraction were left in the filter bag and determined gravimetrically (AACC, 1999; Komarek et al., 1994; USDA, 1970). The results were reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.14. Protein

The protein and other organic nitrogen in the sample were converted to ammonia by digesting the sample with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25 (AOAC, 2011j; k; AOCS, 2009a) The results were reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.15. Total Dietary Fiber

Duplicate samples were gelatinized with α-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The sample was filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fiber in the sample was calculated using protein and ash values (AOAC, 2011j). The results were reported on fresh weight basis. The limit of quantitation was 1.00%.

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 quantified by high-performance liquid chromatography using a silica column (Cort, et al., 1983; McMurray, et al., 1980; Speek, et al., 1985). The results were reported on fresh weight basis. The limit of quantitation was 0.500 mg/100g.

Note: Alpha tocopherol is part of a mixed standard which also includes beta, delta, and gamma isomers. The reference standard material for those isomers may contain small amounts of alpha tocopherol. All reference standards that contributed to the alpha tocopherol concentration are listed below.

Reference Standards:

- USP, Alpha Tocopherol, 98.9%, Lot Number N0F068
- Acros Organics, D-gamma-Tocopherol, 99.4%, A0083534
- Sigma-Aldrich, (+)-delta-Tocopherol, 92%, 090M1916V

E.5. Data Processing and Statistical Analysis

After compositional analyses were performed, data spreadsheets containing individual values for each analysis were sent to Monsanto Company (St. Louis, Missouri) for review. Data were then transferred to Certus International (Chesterfield, MO) where they were converted into the appropriate units and statistically analyzed. The formulas that were used for re-expression of composition data for statistical analysis are listed in Table E-2.

Table E-2. Re-expression Formulas for Statistical Analysis of Composition Data

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture and Calories), Fiber, Gossypol	% fw	% dw	X/d
Calories	Kcal/100g fw	Kcal/100g dw	X/d
Copper, Iron, Manganese, Zinc	ppm fw	mg/kg dw	X/d
Calcium, Magnesium, Phosphorus, Potassium, Sodium	ppm fw	% dw	$X/(10^4 d)$
Vitamin E	mg/100g fw	mg/kg dw	10(X/d)
Amino Acids (AA)	mg/g fw	% dw	X/(10d)
Sterculic, Malvalic, and Dihydrosterculic Acids[2]	µg/g fw	% fw	$X/10^4$
Fatty Acids (FA)	% fw	% Total FA	$(100)X_j/\Sigma X$, for each FA_j where ΣX is over all the FA

[1]d is the fraction of the sample that is dry matter.
[2]Sterculic Acid, Malvalic Acid and Dihydrosterculic Acid were first converted to % fw as an intermediate step for final re-expression as % Total FA.

In order to complete a statistical analysis for a compositional component in this study, 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. The following 13 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, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma-linolenic acid, 20:1 eicosenoic acid; 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid.

If less than 50% of the observations for a component were below the LOQ, individual analyses that were below the LOQ were assigned a value equal to one-half the LOQ. In this study 187 values for 22:0 behenic acid were assigned a value of 0.010% fw and 187 values for sodium were assigned a value of 50.00 ppm fw.

The data were assessed for potential outliers using a studentized PRESS residuals calculation. A PRESS residual is the difference between any value and its value predicted from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme data points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. One sodium value from the commercial control at the ARTI site and one sodium value from a commercial reference

at the ARTI site were extreme data points that were outside the ± 6 studentized PRESS residual range and were removed from the statistical analysis.

All cottonseed components were statistically analyzed using a mixed model analysis of variance. The eight replicated field sites were analyzed individually and as a combined data set. Individual site analysis mean comparison tests were not conducted on site ARTI sodium content because only one Coker 130 replicate was available at that site.

Analyses of the combined replicated sites were performed using model (1).

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

where Y_{ijk} = unique individual observation, U = overall mean, T_i = substance effect, L_j = random site effect, $B(L)_{jk}$ = random block within site effect, LT_{ij} = random site by substance interaction effect, and e_{ijk} = residual error.

Individual sites were also analyzed separately. Individual site analyses were performed using model (2).

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

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

Pairwise comparisons between the test and control materials were defined within the ANOVA and tested using t-tests. The variability from the ANOVA was used to compute the standard error of the difference and to conduct the t-tests for the comparisons.

For each compositional component, a range of observed values and a 99% tolerance interval 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 are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional cotton. Each tolerance interval estimate was based upon the average observation for each unique reference material. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

SAS® (Version 9.2) 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-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Unis)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.62 (0.087) (4.51 - 4.74)	4.74 (0.12) (4.49 - 5.00)	-0.13 (0.15) (-0.49 - 0.047)	-0.55, 0.29	0.440	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	495.41 (3.00) (487.88 - 504.08)	488.30 (3.88) (487.70 - 494.60)	7.11 (3.90) (4.92 - 16.38)	-3.73, 17.94	0.142	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	45.08 (0.58) (43.42 - 46.31)	46.35 (0.81) (45.03 - 47.37)	-1.27 (0.96) (-3.95 - -0.33)	-3.94, 1.39	0.254	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	7.10 (0.27) (6.71 - 7.58)	7.63 (0.35) (7.32 - 7.40)	-0.53 (0.35) (-0.69 - -0.36)	-1.49, 0.43	0.197	4.79, 9.92 (6.05 - 10.50)
Protein	27.53 (0.24) (27.16 - 28.11)	27.04 (0.33) (26.97 - 27.11)	0.49 (0.41) (0.12 - 0.65)	-0.65, 1.63	0.297	22.30, 29.41 (20.58 - 29.28)
Total Fat	22.76 (0.59) (21.32 - 24.40)	21.50 (0.78) (21.15 - 22.89)	1.26 (0.81) (0.69 - 3.25)	-0.98, 3.50	0.193	15.01, 28.51 (16.58 - 25.25)

Table E-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	24.81 (0.32) (24.44 - 25.20)	27.53 (0.45) (26.57 - 28.49)	-2.71 (0.55) (-3.98 - -2.13)	-4.23, -1.20	0.007	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	18.33 (0.90) (15.97 - 20.56)	19.47 (1.20) (19.33 - 19.85)	-1.14 (1.26) (-3.36 - -0.40)	-4.64, 2.36	0.417	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	31.27 (0.79) (29.99 - 32.89)	32.89 (1.06) (30.67 - 34.42)	-1.61 (1.13) (-4.43 - 0.095)	-4.75, 1.53	0.227	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	40.85 (1.06) (39.82 - 42.13)	41.67 (1.50) (40.50 - 42.84)	-0.82 (1.83) (-0.70 - 1.09)	-5.91, 4.27	0.678	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.05 (0.015) (1.01 - 1.09)	1.02 (0.018) (0.99 - 1.05)	0.029 (0.017) (0.011 - 0.036)	-0.018, 0.077	0.161	0.86, 1.11 (0.83 - 1.22)
Arginine	3.00 (0.052) (2.86 - 3.07)	3.02 (0.073) (2.89 - 3.13)	-0.018 (0.084) (-0.098 - -0.032)	-0.25, 0.21	0.840	2.38, 3.47 (2.30 - 3.55)

Table E-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.41 (0.045) (2.29 - 2.48)	2.32 (0.060) (2.19 - 2.42)	0.087 (0.065) (-0.031 - 0.10)	-0.093, 0.27	0.252	1.94, 2.57 (1.79 - 2.72)
Cystine	0.40 (0.010) (0.38 - 0.42)	0.37 (0.014) (0.35 - 0.39)	0.034 (0.017) (0.014 - 0.074)	-0.015, 0.082	0.124	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.82 (0.099) (4.61 - 5.07)	4.51 (0.14) (4.34 - 4.67)	0.31 (0.17) (0.11 - 0.27)	-0.15, 0.78	0.134	3.74, 5.28 (3.39 - 5.45)
Glycine	1.10 (0.020) (1.05 - 1.14)	1.08 (0.026) (1.03 - 1.11)	0.027 (0.028) (-0.015 - 0.021)	-0.052, 0.11	0.397	0.90, 1.14 (0.85 - 1.23)
Histidine	0.74 (0.016) (0.71 - 0.76)	0.74 (0.023) (0.71 - 0.77)	0.0028 (0.026) (-0.0019 - -0.00095)	-0.071, 0.076	0.919	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.89 (0.018) (0.87 - 0.92)	0.91 (0.024) (0.88 - 0.93)	-0.010 (0.025) (-0.027 - -0.0076)	-0.079, 0.058	0.696	0.75, 0.96 (0.72 - 1.03)

Table E-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.53 (0.027) (1.47 - 1.55)	1.50 (0.037) (1.44 - 1.55)	0.029 (0.042) (-0.0060 - 0.032)	-0.086, 0.14	0.524	1.25, 1.62 (1.20 - 1.72)
Lysine	1.22 (0.044) (1.15 - 1.27)	1.23 (0.058) (1.19 - 1.26)	-0.0029 (0.062) (-0.041 - -0.016)	-0.18, 0.17	0.965	1.01, 1.30 (0.99 - 1.44)
Methionine	0.39 (0.015) (0.35 - 0.43)	0.35 (0.021) (0.35 - 0.36)	0.041 (0.025) (-0.014 - 0.087)	-0.029, 0.11	0.181	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.43 (0.027) (1.36 - 1.45)	1.41 (0.038) (1.34 - 1.48)	0.016 (0.045) (-0.027 - 0.022)	-0.11, 0.14	0.737	1.12, 1.58 (1.10 - 1.63)
Proline	0.97 (0.021) (0.95 - 1.00)	1.00 (0.028) (0.98 - 1.02)	-0.027 (0.030) (-0.027 - -0.024)	-0.11, 0.057	0.417	0.83, 1.08 (0.79 - 1.17)
Serine	1.11 (0.022) (1.07 - 1.19)	1.03 (0.031) (0.99 - 1.06)	0.089 (0.038) (0.011 - 0.096)	-0.016, 0.19	0.079	0.83, 1.21 (0.81 - 1.24)

Table E-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.88 (0.012) (0.85 - 0.90)	0.85 (0.017) (0.82 - 0.88)	0.029 (0.021) (-0.0045 - 0.033)	-0.028, 0.087	0.230	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.0062) (0.40 - 0.42)	0.42 (0.0087) (0.40 - 0.44)	-0.012 (0.011) (-0.041 - 0.0060)	-0.042, 0.017	0.306	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.82 (0.015) (0.79 - 0.84)	0.79 (0.021) (0.76 - 0.82)	0.030 (0.026) (0.0011 - 0.028)	-0.042, 0.10	0.313	0.67, 0.84 (0.63 - 0.91)
Valine	1.19 (0.021) (1.14 - 1.23)	1.21 (0.027) (1.17 - 1.24)	-0.018 (0.026) (-0.030 - -0.016)	-0.090, 0.055	0.537	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.77 (0.0085) (0.76 - 0.79)	0.78 (0.012) (0.77 - 0.78)	-0.0049 (0.013) (-0.029 - 0.0018)	-0.041, 0.031	0.723	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	25.16 (0.10) (25.01 - 25.28)	24.98 (0.14) (24.92 - 25.05)	0.18 (0.18) (0.028 - 0.094)	-0.31, 0.67	0.360	16.54, 30.55 (19.11 - 26.73)

Table E-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.53 (0.0052) (0.52 - 0.54)	0.52 (0.0073) (0.52 - 0.52)	0.017 (0.0089) (-0.00060 - 0.017)	-0.0082, 0.041	0.137	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.68 (0.026) (2.65 - 2.72)	2.51 (0.036) (2.45 - 2.57)	0.17 (0.045) (0.083 - 0.22)	0.044, 0.29	0.019	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.81 (0.11) (14.46 - 15.08)	14.68 (0.15) (14.58 - 14.70)	0.12 (0.17) (-0.24 - 0.18)	-0.34, 0.59	0.501	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	54.73 (0.22) (54.24 - 55.29)	55.31 (0.31) (55.26 - 55.38)	-0.59 (0.37) (-0.45 - 0.037)	-1.62, 0.45	0.189	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.14 (0.0021) (0.14 - 0.15)	0.13 (0.0030) (0.12 - 0.14)	0.015 (0.0035) (0.0064 - 0.021)	0.0056, 0.025	0.012	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.31 (0.0079) (0.31 - 0.32)	0.29 (0.011) (0.27 - 0.31)	0.023 (0.011) (0.0032 - 0.046)	-0.0087, 0.054	0.115	0.17, 0.38 (0.20 - 0.36)

Table E-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.14 (0.0026) (0.13 - 0.14)	0.15 (0.0033) (0.15 - 0.16)	-0.015 (0.0031) (-0.019 - -0.012)	-0.024, -0.0065	0.008	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0035) (0.14 - 0.16)	0.12 (0.0050) (0.12 - 0.13)	0.028 (0.0061) (0.024 - 0.035)	0.011, 0.045	0.010	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	9.66 (0.34) (9.23 - 10.15)	9.64 (0.41) (8.79 - 9.79)	0.018 (0.38) (-0.57 - 0.58)	-1.03, 1.06	0.963	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	75.27 (5.63) (72.55 - 77.65)	80.76 (7.78) (72.89 - 87.72)	-5.49 (8.79) (-15.17 - 2.25)	-29.91, 18.93	0.566	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.41 (0.0076) (0.40 - 0.42)	0.40 (0.010) (0.38 - 0.41)	0.0099 (0.011) (0.0077 - 0.016)	-0.020, 0.040	0.413	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	13.27 (0.63) (12.58 - 13.63)	11.50 (0.89) (11.34 - 11.55)	1.77 (1.06) (1.03 - 1.95)	-1.18, 4.73	0.171	9.07, 17.33 (9.07 - 17.14)

Table E-3. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.83 (0.012) (0.82 - 0.84)	0.84 (0.016) (0.82 - 0.87)	-0.015 (0.018) (-0.027 - 0.0054)	-0.066, 0.036	0.450	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.18 (0.029) (1.16 - 1.20)	1.11 (0.040) (1.06 - 1.15)	0.071 (0.047) (0.028 - 0.14)	-0.059, 0.20	0.204	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.027 (0.0038) (0.023 - 0.029)	 (0.013 - 0.013)			ND[6]	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	37.08 (1.65) (35.15 - 38.88)	40.81 (2.03) (38.63 - 40.71)	-3.72 (1.87) (-3.48 - -3.41)	-8.92, 1.47	0.117	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	147.20 (2.44) (144.99 - 149.40)	136.55 (3.46) (133.79 - 139.31)	10.65 (4.23) (6.47 - 15.60)	-1.10, 22.40	0.065	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.
[6]Not determined due to insufficient number of observations for the control.

Table E-4. Statistical Summary of Site ARTI Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0073) (0.14 - 0.16)	0.15 (0.010) (0.14 - 0.15)	0.0022 (0.013) (-0.0021 - 0.00026)	-0.033, 0.037	0.867	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.37 (0.016) (0.34 - 0.39)	0.36 (0.023) (0.33 - 0.37)	0.0088 (0.025) (-0.031 - 0.032)	-0.061, 0.078	0.742	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.21 (0.014) (0.19 - 0.22)	0.20 (0.020) (0.19 - 0.20)	0.013 (0.023) (-0.0076 - 0.032)	-0.052, 0.078	0.605	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.91 (0.035) (0.80 - 1.02)	0.82 (0.049) (0.80 - 0.84)	0.090 (0.060) (-0.035 - 0.10)	-0.076, 0.26	0.207	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.97 (0.034) (0.89 - 1.04)	0.93 (0.044) (0.94 - 0.98)	0.042 (0.044) (0.0013 - 0.10)	-0.081, 0.16	0.399	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.53 (0.044) (4.45 - 4.57)	4.21 (0.047) (4.12 - 4.23)	0.32 (0.034) (0.31 - 0.34)	0.23, 0.41	<0.001	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	497.72 (2.28) (489.91 - 504.20)	496.55 (2.63) (494.57 - 498.27)	1.16 (3.48) (-6.91 - 8.49)	-7.79, 10.12	0.751	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.91 (0.59) (43.42 - 46.94)	45.84 (0.68) (44.64 - 47.09)	-0.94 (0.91) (-3.23 - 1.14)	-3.27, 1.39	0.348	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.98 (0.15) (6.42 - 7.33)	7.23 (0.18) (6.99 - 7.48)	-0.25 (0.23) (-1.06 - 0.12)	-0.85, 0.35	0.328	4.79, 9.92 (6.05 - 10.50)
Protein	27.41 (0.33) (26.87 - 27.78)	27.30 (0.37) (26.45 - 28.21)	0.11 (0.37) (-0.43 - 0.96)	-0.83, 1.06	0.770	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.14 (0.46) (21.58 - 24.46)	22.67 (0.53) (22.26 - 23.02)	0.47 (0.70) (-1.16 - 1.93)	-1.34, 2.28	0.536	15.01, 28.51 (16.58 - 25.25)

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.69 (0.52) (25.04 - 26.77)	27.52 (0.60) (26.81 - 28.13)	-1.83 (0.79) (-3.09 - -0.87)	-3.86, 0.20	0.068	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	19.27 (0.68) (17.10 - 20.64)	19.92 (0.78) (18.70 - 21.18)	-0.64 (1.00) (-1.60 - -0.54)	-3.21, 1.92	0.547	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	31.47 (0.90) (29.71 - 34.04)	33.92 (1.04) (32.79 - 35.89)	-2.44 (1.38) (-5.13 - -1.40)	-5.98, 1.09	0.135	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.64 (0.86) (37.72 - 41.91)	41.11 (1.00) (39.89 - 42.04)	-1.46 (1.32) (-4.32 - -0.44)	-4.85, 1.93	0.318	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.04 (0.019) (1.02 - 1.05)	1.10 (0.022) (1.06 - 1.17)	-0.059 (0.025) (-0.13 - -0.016)	-0.12, 0.0050	0.064	0.86, 1.11 (0.83 - 1.22)
Arginine	2.95 (0.061) (2.87 - 3.02)	3.21 (0.067) (3.07 - 3.46)	-0.27 (0.064) (-0.47 - -0.14)	-0.43, -0.10	0.008	2.38, 3.47 (2.30 - 3.55)

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.31 (0.043) (2.24 - 2.36)	2.45 (0.047) (2.36 - 2.60)	-0.15 (0.044) (-0.29 - -0.054)	-0.26, -0.035	0.019	1.94, 2.57 (1.79 - 2.72)
Cystine	0.40 (0.012) (0.38 - 0.42)	0.40 (0.013) (0.38 - 0.43)	-0.0089 (0.018) (-0.052 - 0.011)	-0.054, 0.036	0.636	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.57 (0.098) (4.35 - 4.77)	4.96 (0.11) (4.77 - 5.21)	-0.39 (0.099) (-0.63 - -0.17)	-0.65, -0.14	0.010	3.74, 5.28 (3.39 - 5.45)
Glycine	1.08 (0.020) (1.06 - 1.12)	1.13 (0.023) (1.09 - 1.20)	-0.052 (0.026) (-0.13 - 0.011)	-0.12, 0.014	0.099	0.90, 1.14 (0.85 - 1.23)
Histidine	0.73 (0.013) (0.68 - 0.76)	0.76 (0.014) (0.75 - 0.78)	-0.029 (0.012) (-0.022 - -0.020)	-0.061, 0.0030	0.067	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.90 (0.010) (0.90 - 0.91)	0.94 (0.012) (0.92 - 0.97)	-0.040 (0.014) (-0.074 - -0.012)	-0.075, -0.0042	0.034	0.75, 0.96 (0.72 - 1.03)

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.51 (0.022) (1.49 - 1.54)	1.58 (0.024) (1.52 - 1.65)	-0.068 (0.021) (-0.14 - -0.030)	-0.12, -0.013	0.024	1.25, 1.62 (1.20 - 1.72)
Lysine	1.24 (0.018) (1.21 - 1.28)	1.23 (0.020) (1.22 - 1.25)	0.0073 (0.024) (0.0016 - 0.032)	-0.055, 0.069	0.775	1.01, 1.30 (0.99 - 1.44)
Methionine	0.40 (0.017) (0.36 - 0.43)	0.42 (0.019) (0.37 - 0.46)	-0.025 (0.024) (-0.057 - -0.011)	-0.086, 0.036	0.337	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.40 (0.030) (1.37 - 1.43)	1.49 (0.033) (1.41 - 1.61)	-0.088 (0.032) (-0.18 - -0.033)	-0.17, -0.0064	0.039	1.12, 1.58 (1.10 - 1.63)
Proline	0.98 (0.020) (0.97 - 0.99)	1.05 (0.022) (1.03 - 1.09)	-0.065 (0.022) (-0.097 - -0.032)	-0.12, -0.0075	0.033	0.83, 1.08 (0.79 - 1.17)
Serine	1.03 (0.031) (0.96 - 1.11)	1.12 (0.035) (1.08 - 1.20)	-0.090 (0.039) (-0.18 - 0.011)	-0.19, 0.010	0.069	0.83, 1.21 (0.81 - 1.24)

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.85 (0.015) (0.83 - 0.88)	0.90 (0.017) (0.87 - 0.95)	-0.046 (0.018) (-0.10 - -0.0034)	-0.092, -0.00006	0.049	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.42 (0.011) (0.39 - 0.45)	0.42 (0.013) (0.39 - 0.44)	0.0013 (0.017) (-0.044 - 0.041)	-0.043, 0.046	0.942	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.80 (0.011) (0.79 - 0.82)	0.84 (0.013) (0.81 - 0.89)	-0.036 (0.013) (-0.068 - -0.015)	-0.069, -0.0030	0.037	0.67, 0.84 (0.63 - 0.91)
Valine	1.21 (0.018) (1.19 - 1.23)	1.26 (0.019) (1.23 - 1.32)	-0.053 (0.015) (-0.090 - -0.022)	-0.091, -0.014	0.017	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.78 (0.0068) (0.76 - 0.79)	0.77 (0.0079) (0.76 - 0.78)	0.0029 (0.010) (-0.026 - 0.018)	-0.024, 0.030	0.793	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.40 (0.14) (24.27 - 24.66)	24.12 (0.16) (23.78 - 24.45)	0.28 (0.20) (-0.11 - 0.56)	-0.24, 0.80	0.227	16.54, 30.55 (19.11 - 26.73)

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.52 (0.0058) (0.51 - 0.54)	0.51 (0.0067) (0.50 - 0.52)	0.011 (0.0089) (-0.012 - 0.025)	-0.012, 0.034	0.268	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.54 (0.032) (2.45 - 2.67)	2.43 (0.037) (2.37 - 2.46)	0.11 (0.046) (0.066 - 0.21)	-0.0057, 0.23	0.058	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.64 (0.098) (14.39 - 14.84)	14.39 (0.11) (14.06 - 14.61)	0.25 (0.13) (-0.0098 - 0.34)	-0.078, 0.58	0.107	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.81 (0.24) (55.04 - 56.24)	56.59 (0.28) (56.02 - 57.32)	-0.78 (0.35) (-1.39 - 0.12)	-1.67, 0.12	0.075	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.15 (0.0066) (0.15 - 0.16)	0.15 (0.0076) (0.14 - 0.15)	0.0076 (0.010) (0.00091 - 0.013)	-0.018, 0.033	0.481	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.29 (0.0098) (0.26 - 0.31)	0.28 (0.011) (0.26 - 0.29)	0.013 (0.013) (0.0016 - 0.015)	-0.021, 0.047	0.376	0.17, 0.38 (0.20 - 0.36)

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.14 (0.0026) (0.14 - 0.15)	0.14 (0.0030) (0.14 - 0.15)	-0.00014 (0.0037) (-0.0017 - 0.0028)	-0.0097, 0.0095	0.971	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.13 (0.0015) (0.13 - 0.13)	0.11 (0.0018) (0.11 - 0.11)	0.019 (0.0023) (0.014 - 0.024)	0.013, 0.025	<0.001	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	8.51 (0.26) (8.02 - 9.13)	8.21 (0.30) (7.48 - 8.64)	0.30 (0.39) (-0.49 - 1.13)	-0.70, 1.31	0.473	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	75.42 (3.80) (70.35 - 79.72)	78.00 (4.39) (75.01 - 80.40)	-2.58 (5.81) (-8.31 - 4.71)	-17.51, 12.35	0.675	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.41 (0.0052) (0.40 - 0.41)	0.38 (0.0054) (0.37 - 0.39)	0.026 (0.0034) (0.021 - 0.031)	0.018, 0.035	<0.001	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	13.41 (0.31) (12.79 - 14.14)	11.51 (0.34) (10.81 - 11.75)	1.90 (0.37) (1.18 - 2.39)	0.95, 2.85	0.003	9.07, 17.33 (9.07 - 17.14)

Table E-5. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.78 (0.011) (0.75 - 0.81)	0.76 (0.012) (0.75 - 0.79)	0.018 (0.0082) (0.0052 - 0.029)	-0.0028, 0.040	0.076	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.21 (0.012) (1.17 - 1.24)	1.12 (0.013) (1.10 - 1.13)	0.090 (0.010) (0.075 - 0.11)	0.064, 0.12	<0.001	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.022 (0.0045) (0.019 - 0.027)	0.017 (0.0052) (0.013 - 0.022)	0.0049 (0.0069) (-0.0020 - 0.014)	-0.013, 0.023	0.515	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	39.10 (0.67) (37.41 - 40.18)	39.55 (0.75) (38.49 - 40.84)	-0.45 (0.83) (-1.35 - 0.51)	-2.58, 1.68	0.610	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	151.03 (2.27) (148.34 - 154.95)	140.12 (2.63) (133.64 - 145.15)	10.90 (3.47) (3.19 - 18.52)	1.97, 19.83	0.025	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-6. Statistical Summary of Site GACH Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0067) (0.14 - 0.16)	0.12 (0.0077) (0.11 - 0.13)	0.033 (0.010) (0.021 - 0.050)	0.0069, 0.059	0.022	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.37 (0.027) (0.26 - 0.45)	0.32 (0.031) (0.31 - 0.34)	0.044 (0.038) (-0.052 - 0.12)	-0.055, 0.14	0.304	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.21 (0.011) (0.18 - 0.25)	0.18 (0.013) (0.18 - 0.20)	0.030 (0.015) (-0.0018 - 0.050)	-0.0072, 0.068	0.092	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.85 (0.017) (0.83 - 0.88)	0.86 (0.019) (0.85 - 0.90)	-0.016 (0.020) (-0.055 - 0.028)	-0.068, 0.037	0.474	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.97 (0.019) (0.93 - 1.01)	0.96 (0.020) (0.90 - 0.99)	0.019 (0.017) (-0.012 - 0.033)	-0.026, 0.063	0.324	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.53 (0.063) (4.25 - 4.66)	4.29 (0.071) (4.21 - 4.33)	0.24 (0.078) (0.042 - 0.34)	0.040, 0.44	0.027	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	496.63 (2.75) (492.91 - 499.03)	495.83 (3.16) (492.30 - 504.10)	0.80 (3.87) (-8.23 - 6.50)	-9.15, 10.75	0.844	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.10 (0.71) (42.20 - 44.98)	44.23 (0.82) (42.53 - 45.14)	-0.14 (1.09) (-0.77 - 2.45)	-2.94, 2.67	0.905	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	7.02 (0.17) (6.63 - 7.36)	7.36 (0.19) (7.17 - 7.62)	-0.34 (0.20) (-0.73 - 0.19)	-0.86, 0.18	0.153	4.79, 9.92 (6.05 - 10.50)
Protein	28.42 (0.62) (26.95 - 30.82)	28.82 (0.72) (28.58 - 29.04)	-0.40 (0.93) (-1.63 - -0.87)	-2.80, 1.99	0.681	22.30, 29.41 (20.58 - 29.28)
Total Fat	22.96 (0.55) (22.34 - 23.50)	22.62 (0.64) (21.87 - 24.18)	0.34 (0.81) (-1.58 - 1.56)	-1.75, 2.43	0.692	15.01, 28.51 (16.58 - 25.25)

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	24.04 (0.55) (23.86 - 24.16)	24.01 (0.61) (22.08 - 25.22)	0.027 (0.65) (-1.08 - 1.77)	-1.64, 1.70	0.968	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	16.43 (0.24) (16.06 - 17.24)	17.67 (0.28) (17.49 - 17.88)	-1.24 (0.37) (-1.69 - -0.24)	-2.19, -0.29	0.019	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	28.04 (0.88) (25.13 - 30.18)	30.20 (1.01) (28.87 - 32.60)	-2.15 (1.21) (-2.93 - 0.63)	-5.27, 0.97	0.136	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	38.32 (0.42) (37.62 - 38.75)	40.14 (0.49) (39.32 - 41.35)	-1.83 (0.63) (-3.74 - -0.57)	-3.46, -0.19	0.034	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.04 (0.011) (1.02 - 1.05)	1.06 (0.012) (1.02 - 1.10)	-0.022 (0.014) (-0.048 - -0.00010)	-0.058, 0.014	0.175	0.86, 1.11 (0.83 - 1.22)
Arginine	3.02 (0.053) (2.95 - 3.10)	3.28 (0.060) (3.10 - 3.43)	-0.26 (0.069) (-0.34 - -0.14)	-0.43, -0.082	0.013	2.38, 3.47 (2.30 - 3.55)

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.38 (0.037) (2.32 - 2.46)	2.50 (0.043) (2.39 - 2.64)	-0.11 (0.053) (-0.26 - -0.073)	-0.25, 0.022	0.083	1.94, 2.57 (1.79 - 2.72)
Cystine	0.43 (0.010) (0.41 - 0.44)	0.42 (0.012) (0.41 - 0.43)	0.0076 (0.016) (-0.017 - 0.015)	-0.033, 0.048	0.651	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.82 (0.12) (4.58 - 5.22)	5.07 (0.14) (4.86 - 5.43)	-0.25 (0.18) (-0.78 - -0.017)	-0.71, 0.21	0.219	3.74, 5.28 (3.39 - 5.45)
Glycine	1.08 (0.018) (1.06 - 1.12)	1.11 (0.021) (1.07 - 1.18)	-0.029 (0.027) (-0.11 - -0.0024)	-0.099, 0.040	0.330	0.90, 1.14 (0.85 - 1.23)
Histidine	0.75 (0.013) (0.73 - 0.77)	0.77 (0.014) (0.74 - 0.80)	-0.024 (0.011) (-0.045 - 0.0022)	-0.052, 0.0035	0.074	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.92 (0.014) (0.91 - 0.95)	0.95 (0.015) (0.91 - 0.98)	-0.027 (0.014) (-0.062 - 0.0079)	-0.063, 0.0091	0.112	0.75, 0.96 (0.72 - 1.03)

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.54 (0.020) (1.51 - 1.57)	1.58 (0.022) (1.52 - 1.65)	-0.043 (0.021) (-0.083 - -0.0065)	-0.097, 0.011	0.098	1.25, 1.62 (1.20 - 1.72)
Lysine	1.22 (0.017) (1.21 - 1.23)	1.25 (0.019) (1.19 - 1.30)	-0.033 (0.021) (-0.073 - 0.027)	-0.087, 0.021	0.178	1.01, 1.30 (0.99 - 1.44)
Methionine	0.39 (0.015) (0.37 - 0.42)	0.39 (0.017) (0.34 - 0.44)	0.0021 (0.019) (-0.017 - 0.027)	-0.046, 0.050	0.915	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.44 (0.022) (1.40 - 1.46)	1.53 (0.025) (1.45 - 1.58)	-0.090 (0.029) (-0.13 - -0.049)	-0.16, -0.016	0.025	1.12, 1.58 (1.10 - 1.63)
Proline	1.01 (0.018) (0.98 - 1.03)	1.07 (0.020) (1.03 - 1.12)	-0.060 (0.027) (-0.12 - -0.042)	-0.13, 0.0090	0.075	0.83, 1.08 (0.79 - 1.17)
Serine	1.08 (0.029) (1.03 - 1.18)	1.11 (0.034) (1.06 - 1.20)	-0.031 (0.045) (-0.17 - 0.0023)	-0.15, 0.083	0.513	0.83, 1.21 (0.81 - 1.24)

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.86 (0.013) (0.84 - 0.88)	0.88 (0.015) (0.83 - 0.92)	-0.022 (0.019) (-0.053 - 0.0050)	-0.071, 0.028	0.317	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.42 (0.0061) (0.42 - 0.43)	0.42 (0.0070) (0.42 - 0.43)	-0.0028 (0.0090) (-0.016 - 0.011)	-0.026, 0.020	0.771	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.80 (0.014) (0.78 - 0.83)	0.84 (0.016) (0.79 - 0.87)	-0.039 (0.019) (-0.074 - -0.015)	-0.087, 0.0096	0.094	0.67, 0.84 (0.63 - 0.91)
Valine	1.21 (0.017) (1.19 - 1.23)	1.26 (0.019) (1.21 - 1.30)	-0.048 (0.023) (-0.085 - -0.027)	-0.11, 0.010	0.087	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.68 (0.0087) (0.66 - 0.71)	0.72 (0.0096) (0.71 - 0.73)	-0.038 (0.0090) (-0.049 - -0.016)	-0.061, -0.015	0.007	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	22.61 (0.089) (22.34 - 22.84)	22.73 (0.10) (22.69 - 22.78)	-0.12 (0.13) (-0.11 - 0.093)	-0.46, 0.21	0.394	16.54, 30.55 (19.11 - 26.73)

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.50 (0.0057) (0.48 - 0.52)	0.49 (0.0062) (0.49 - 0.50)	0.0067 (0.0059) (-0.0046 - 0.021)	-0.0086, 0.022	0.312	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.31 (0.031) (2.29 - 2.32)	2.20 (0.036) (2.15 - 2.28)	0.11 (0.047) (0.039 - 0.17)	-0.016, 0.23	0.075	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.69 (0.11) (14.51 - 14.88)	14.83 (0.13) (14.74 - 14.99)	-0.14 (0.17) (-0.48 - 0.14)	-0.59, 0.31	0.454	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	57.84 (0.19) (57.49 - 58.22)	57.78 (0.22) (57.65 - 57.93)	0.059 (0.29) (-0.44 - 0.19)	-0.67, 0.79	0.843	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.18 (0.0027) (0.18 - 0.19)	0.17 (0.0030) (0.17 - 0.18)	0.0066 (0.0033) (0.0051 - 0.010)	-0.0018, 0.015	0.100	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.23 (0.0029) (0.23 - 0.24)	0.24 (0.0034) (0.23 - 0.24)	-0.0021 (0.0045) (-0.0063 - 0.0087)	-0.014, 0.0094	0.651	0.17, 0.38 (0.20 - 0.36)

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.13 (0.0030) (0.12 - 0.14)	0.13 (0.0035) (0.13 - 0.14)	-0.0051 (0.0046) (-0.0084 - 0.0036)	-0.017, 0.0067	0.318	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.20 (0.0060) (0.19 - 0.22)	0.18 (0.0065) (0.17 - 0.19)	0.026 (0.0061) (0.013 - 0.038)	0.010, 0.042	0.007	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	10.08 (0.54) (8.74 - 11.06)	11.01 (0.61) (10.09 - 11.33)	-0.93 (0.71) (-2.59 - 0.69)	-2.75, 0.89	0.246	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	78.87 (5.50) (74.42 - 82.99)	74.39 (6.35) (72.65 - 76.27)	4.48 (8.40) (3.93 - 8.73)	-17.11, 26.07	0.616	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.43 (0.0033) (0.41 - 0.43)	0.40 (0.0038) (0.39 - 0.40)	0.027 (0.0046) (0.015 - 0.041)	0.015, 0.039	0.002	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	13.34 (0.29) (12.74 - 13.60)	12.56 (0.33) (11.96 - 13.28)	0.78 (0.44) (-0.53 - 1.64)	-0.35, 1.90	0.135	9.07, 17.33 (9.07 - 17.14)

Table E-7. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.79 (0.0090) (0.75 - 0.82)	0.78 (0.010) (0.77 - 0.79)	0.0090 (0.011) (-0.024 - 0.029)	-0.019, 0.037	0.440	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.09 (0.010) (1.08 - 1.11)	1.08 (0.012) (1.05 - 1.10)	0.018 (0.015) (-0.0048 - 0.063)	-0.020, 0.056	0.281	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.022 (0.0028) (0.019 - 0.025)	0.0080 (0.0032) (0.0054 - 0.013)	0.014 (0.0042) (0.0098 - 0.016)	0.0033, 0.025	0.020	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	40.79 (1.18) (37.59 - 43.87)	42.00 (1.37) (40.59 - 43.50)	-1.21 (1.81) (-5.91 - 3.27)	-5.85, 3.44	0.533	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	93.11 (2.67) (86.23 - 100.03)	92.34 (2.91) (91.78 - 95.85)	0.76 (2.71) (-6.54 - 4.18)	-6.20, 7.72	0.789	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-8. Statistical Summary of Site KSLA Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.010) (0.11 - 0.18)	0.12 (0.012) (0.12 - 0.13)	0.026 (0.014) (-0.0087 - 0.054)	-0.011, 0.063	0.129	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.45 (0.030) (0.34 - 0.55)	0.37 (0.034) (0.33 - 0.39)	0.083 (0.039) (-0.024 - 0.15)	-0.018, 0.18	0.088	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.014) (0.18 - 0.28)	0.20 (0.016) (0.19 - 0.21)	0.031 (0.019) (-0.023 - 0.078)	-0.017, 0.079	0.159	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	1.07 (0.027) (1.03 - 1.10)	0.95 (0.030) (0.86 - 1.05)	0.12 (0.033) (0.051 - 0.20)	0.036, 0.20	0.014	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.13 (0.033) (1.00 - 1.24)	1.01 (0.038) (1.00 - 1.02)	0.12 (0.047) (0.0061 - 0.23)	0.00016, 0.24	0.049	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.35 (0.047) (4.23 - 4.47)	4.12 (0.047) (4.06 - 4.15)	0.23 (0.066) (0.11 - 0.41)	0.066, 0.39	0.013	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	494.11 (3.01) (482.46 - 501.83)	494.75 (3.01) (490.27 - 498.67)	-0.64 (4.19) (-14.30 - 4.88)	-10.89, 9.61	0.883	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	45.92 (0.79) (44.17 - 48.89)	47.84 (0.79) (46.77 - 50.30)	-1.91 (1.11) (-5.19 - 1.91)	-4.64, 0.81	0.136	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.70 (0.26) (6.46 - 6.94)	6.98 (0.26) (6.15 - 7.40)	-0.28 (0.36) (-0.90 - 0.79)	-1.17, 0.62	0.478	4.79, 9.92 (6.05 - 10.50)
Protein	27.44 (0.56) (27.06 - 27.92)	25.80 (0.56) (23.53 - 27.85)	1.64 (0.80) (0.074 - 3.73)	-0.31, 3.59	0.084	22.30, 29.41 (20.58 - 29.28)
Total Fat	22.27 (0.64) (19.79 - 23.86)	22.25 (0.64) (21.29 - 23.02)	0.018 (0.90) (-2.89 - 1.18)	-2.18, 2.22	0.984	15.01, 28.51 (16.58 - 25.25)

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.72 (0.45) (24.16 - 27.08)	28.35 (0.45) (27.81 - 29.58)	-2.63 (0.63) (-5.42 - -0.73)	-4.18, -1.08	0.005	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	18.73 (0.66) (17.75 - 19.77)	19.62 (0.66) (18.46 - 20.54)	-0.89 (0.93) (-1.79 - 0.17)	-3.15, 1.38	0.376	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	33.12 (0.65) (32.24 - 34.42)	34.05 (0.65) (32.61 - 35.84)	-0.93 (0.42) (-1.65 - 0.23)	-1.97, 0.10	0.070	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.82 (0.49) (39.02 - 40.86)	43.35 (0.49) (42.33 - 44.37)	-3.53 (0.69) (-5.34 - -1.47)	-5.21, -1.85	0.002	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.07 (0.018) (1.00 - 1.11)	1.03 (0.018) (1.00 - 1.06)	0.038 (0.014) (0.00025 - 0.082)	0.0050, 0.072	0.030	0.86, 1.11 (0.83 - 1.22)
Arginine	2.96 (0.073) (2.64 - 3.13)	2.98 (0.073) (2.89 - 3.13)	-0.017 (0.084) (-0.25 - 0.15)	-0.22, 0.19	0.849	2.38, 3.47 (2.30 - 3.55)

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.37 (0.050) (2.17 - 2.49)	2.30 (0.050) (2.25 - 2.40)	0.074 (0.062) (-0.12 - 0.22)	-0.077, 0.22	0.278	1.94, 2.57 (1.79 - 2.72)
Cystine	0.41 (0.016) (0.38 - 0.46)	0.38 (0.016) (0.36 - 0.44)	0.026 (0.019) (-0.024 - 0.082)	-0.021, 0.074	0.222	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.78 (0.13) (4.32 - 5.26)	4.52 (0.13) (4.37 - 4.75)	0.27 (0.16) (-0.15 - 0.79)	-0.13, 0.67	0.151	3.74, 5.28 (3.39 - 5.45)
Glycine	1.11 (0.020) (1.02 - 1.18)	1.06 (0.020) (1.04 - 1.09)	0.057 (0.025) (-0.024 - 0.14)	-0.0034, 0.12	0.060	0.90, 1.14 (0.85 - 1.23)
Histidine	0.74 (0.020) (0.68 - 0.78)	0.72 (0.020) (0.67 - 0.76)	0.019 (0.014) (0.013 - 0.029)	-0.014, 0.052	0.206	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.90 (0.018) (0.84 - 0.97)	0.88 (0.018) (0.87 - 0.90)	0.020 (0.020) (-0.028 - 0.096)	-0.028, 0.068	0.355	0.75, 0.96 (0.72 - 1.03)

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control)			Commercial Tolerance Interval[5] (Range)
			Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	
Amino Acid (% dw)						
Leucine	1.51 (0.026) (1.40 - 1.58)	1.48 (0.026) (1.44 - 1.52)	0.039 (0.028) (-0.035 - 0.12)	-0.030, 0.11	0.217	1.25, 1.62 (1.20 - 1.72)
Lysine	1.26 (0.024) (1.17 - 1.31)	1.18 (0.024) (1.12 - 1.23)	0.083 (0.027) (0.021 - 0.15)	0.016, 0.15	0.023	1.01, 1.30 (0.99 - 1.44)
Methionine	0.42 (0.017) (0.37 - 0.44)	0.38 (0.017) (0.32 - 0.42)	0.045 (0.013) (0.020 - 0.075)	0.013, 0.077	0.013	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.41 (0.027) (1.28 - 1.47)	1.39 (0.027) (1.36 - 1.43)	0.015 (0.033) (-0.077 - 0.094)	-0.066, 0.095	0.668	1.12, 1.58 (1.10 - 1.63)
Proline	1.00 (0.015) (0.94 - 1.04)	0.98 (0.015) (0.95 - 1.02)	0.017 (0.022) (-0.039 - 0.093)	-0.036, 0.071	0.459	0.83, 1.08 (0.79 - 1.17)
Serine	1.07 (0.022) (0.99 - 1.15)	1.03 (0.022) (1.01 - 1.06)	0.038 (0.030) (-0.047 - 0.12)	-0.036, 0.11	0.257	0.83, 1.21 (0.81 - 1.24)

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.87 (0.016) (0.80 - 0.90)	0.84 (0.016) (0.82 - 0.86)	0.027 (0.012) (-0.021 - 0.060)	-0.0036, 0.057	0.074	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.014) (0.36 - 0.46)	0.39 (0.014) (0.38 - 0.43)	0.019 (0.018) (-0.024 - 0.078)	-0.026, 0.063	0.347	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.80 (0.018) (0.72 - 0.84)	0.79 (0.018) (0.76 - 0.81)	0.010 (0.021) (-0.045 - 0.058)	-0.042, 0.063	0.643	0.67, 0.84 (0.63 - 0.91)
Valine	1.21 (0.024) (1.11 - 1.29)	1.18 (0.024) (1.17 - 1.19)	0.026 (0.029) (-0.054 - 0.12)	-0.044, 0.096	0.397	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.74 (0.013) (0.71 - 0.76)	0.75 (0.013) (0.73 - 0.78)	-0.012 (0.018) (-0.032 - 0.0064)	-0.057, 0.032	0.523	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.48 (0.091) (24.37 - 24.55)	24.04 (0.091) (23.92 - 24.16)	0.44 (0.13) (0.21 - 0.61)	0.12, 0.75	0.014	16.54, 30.55 (19.11 - 26.73)

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.51 (0.0035) (0.50 - 0.52)	0.50 (0.0035) (0.49 - 0.51)	0.0090 (0.0049) (-0.0051 - 0.020)	-0.0030, 0.021	0.116	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.68 (0.018) (2.64 - 2.73)	2.52 (0.018) (2.49 - 2.57)	0.15 (0.026) (0.11 - 0.24)	0.089, 0.22	0.001	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.70 (0.095) (14.48 - 15.01)	14.29 (0.095) (14.13 - 14.53)	0.41 (0.13) (0.16 - 0.72)	0.084, 0.74	0.021	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.53 (0.14) (55.15 - 55.99)	56.63 (0.14) (56.52 - 56.72)	-1.09 (0.20) (-1.42 - -0.63)	-1.59, -0.60	0.001	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.15 (0.0042) (0.14 - 0.17)	0.15 (0.0042) (0.13 - 0.15)	0.0063 (0.0059) (-0.0073 - 0.019)	-0.0082, 0.021	0.327	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.31 (0.0054) (0.31 - 0.32)	0.29 (0.0054) (0.29 - 0.30)	0.020 (0.0071) (0.016 - 0.023)	0.0022, 0.037	0.033	0.17, 0.38 (0.20 - 0.36)

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.14 (0.0039) (0.14 - 0.15)	0.14 (0.0039) (0.14 - 0.15)	0.00029 (0.0055) (-0.0046 - 0.0027)	-0.013, 0.014	0.959	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.12 (0.0039) (0.11 - 0.13)	0.12 (0.0039) (0.12 - 0.12)	0.0047 (0.0050) (-0.012 - 0.015)	-0.0075, 0.017	0.381	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	8.48 (0.30) (7.86 - 9.49)	8.70 (0.30) (8.11 - 9.14)	-0.22 (0.42) (-1.27 - 0.99)	-1.26, 0.82	0.621	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	76.74 (4.19) (73.99 - 83.17)	68.59 (4.19) (66.28 - 70.38)	8.15 (4.72) (3.68 - 12.79)	-3.40, 19.69	0.134	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.41 (0.0065) (0.39 - 0.44)	0.39 (0.0065) (0.38 - 0.41)	0.021 (0.0093) (-0.014 - 0.054)	-0.0018, 0.043	0.065	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	13.13 (0.37) (11.92 - 13.79)	12.87 (0.37) (12.31 - 13.87)	0.26 (0.52) (-1.95 - 1.16)	-1.03, 1.54	0.642	9.07, 17.33 (9.07 - 17.14)

Table E-9. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.75 (0.014) (0.71 - 0.80)	0.71 (0.014) (0.69 - 0.73)	0.036 (0.019) (-0.023 - 0.11)	-0.012, 0.083	0.113	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.18 (0.022) (1.16 - 1.22)	1.17 (0.022) (1.12 - 1.27)	0.0099 (0.029) (-0.087 - 0.064)	-0.061, 0.081	0.742	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.023 (0.0043) (0.021 - 0.024)	0.015 (0.0043) (0.0053 - 0.027)	0.0078 (0.0060) (-0.0031 - 0.017)	-0.0069, 0.022	0.242	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	33.97 (0.93) (31.68 - 37.84)	35.74 (0.93) (35.10 - 37.09)	-1.77 (1.32) (-5.41 - 2.55)	-4.99, 1.45	0.227	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	169.88 (2.48) (163.34 - 175.33)	149.96 (2.48) (148.96 - 152.67)	19.92 (3.48) (14.16 - 26.36)	11.40, 28.43	0.001	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-10. Statistical Summary of Site LACH Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.011) (0.13 - 0.19)	0.13 (0.011) (0.12 - 0.14)	0.019 (0.016) (-0.0082 - 0.068)	-0.019, 0.057	0.271	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.39 (0.035) (0.33 - 0.53)	0.35 (0.035) (0.31 - 0.38)	0.032 (0.049) (-0.047 - 0.16)	-0.088, 0.15	0.535	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.22 (0.016) (0.19 - 0.29)	0.20 (0.016) (0.17 - 0.21)	0.020 (0.023) (-0.011 - 0.076)	-0.037, 0.076	0.432	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.86 (0.026) (0.81 - 0.92)	0.81 (0.026) (0.78 - 0.84)	0.056 (0.036) (0.0028 - 0.13)	-0.033, 0.14	0.175	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.93 (0.025) (0.90 - 1.00)	0.90 (0.025) (0.82 - 0.94)	0.033 (0.036) (-0.015 - 0.086)	-0.055, 0.12	0.395	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.34 (0.057) (4.29 - 4.40)	4.14 (0.064) (3.94 - 4.26)	0.20 (0.070) (0.13 - 0.35)	0.024, 0.39	0.033	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	497.98 (1.61) (491.46 - 501.77)	491.80 (1.86) (488.93 - 494.48)	6.18 (2.46) (-3.01 - 10.35)	-0.15, 12.51	0.053	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.40 (0.59) (43.84 - 45.31)	44.36 (0.68) (43.65 - 45.15)	0.044 (0.87) (-1.31 - 0.79)	-2.20, 2.29	0.961	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	9.18 (0.22) (8.64 - 9.67)	8.96 (0.23) (8.59 - 9.19)	0.22 (0.19) (-0.050 - 0.48)	-0.26, 0.70	0.287	4.79, 9.92 (6.05 - 10.50)
Protein	28.24 (0.61) (26.53 - 29.33)	29.84 (0.70) (29.62 - 30.42)	-1.60 (0.85) (-1.99 - -0.53)	-3.78, 0.59	0.119	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.04 (0.30) (21.89 - 23.76)	21.59 (0.34) (21.03 - 22.21)	1.45 (0.46) (-0.32 - 2.22)	0.28, 2.63	0.024	15.01, 28.51 (16.58 - 25.25)

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.91 (1.12) (24.26 - 27.74)	26.31 (1.29) (24.72 - 28.08)	-0.40 (1.71) (-1.88 - -0.096)	-4.80, 3.99	0.822	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.01 (0.29) (16.31 - 17.78)	16.93 (0.33) (16.30 - 17.90)	0.086 (0.38) (-0.12 - 0.31)	-0.90, 1.08	0.831	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	31.08 (1.03) (29.23 - 32.66)	31.14 (1.19) (30.85 - 31.49)	-0.057 (1.58) (-1.62 - 1.16)	-4.11, 4.00	0.972	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	38.51 (0.55) (36.91 - 39.40)	39.52 (0.64) (39.05 - 39.86)	-1.01 (0.85) (-0.90 - -0.088)	-3.19, 1.16	0.285	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.11 (0.020) (1.07 - 1.14)	1.10 (0.023) (1.05 - 1.14)	0.011 (0.031) (-0.0051 - 0.026)	-0.068, 0.091	0.727	0.86, 1.11 (0.83 - 1.22)
Arginine	3.08 (0.052) (2.97 - 3.20)	3.20 (0.060) (3.11 - 3.27)	-0.12 (0.080) (-0.19 - -0.031)	-0.33, 0.080	0.178	2.38, 3.47 (2.30 - 3.55)

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.50 (0.053) (2.43 - 2.58)	2.55 (0.061) (2.46 - 2.63)	-0.045 (0.081) (-0.042 - 0.0096)	-0.25, 0.16	0.604	1.94, 2.57 (1.79 - 2.72)
Cystine	0.43 (0.018) (0.40 - 0.46)	0.42 (0.021) (0.39 - 0.46)	0.016 (0.028) (-0.051 - 0.055)	-0.056, 0.088	0.594	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.71 (0.13) (4.64 - 4.79)	5.08 (0.15) (4.85 - 5.40)	-0.37 (0.20) (-0.66 - -0.19)	-0.88, 0.14	0.120	3.74, 5.28 (3.39 - 5.45)
Glycine	1.11 (0.019) (1.06 - 1.16)	1.11 (0.022) (1.08 - 1.14)	0.0013 (0.029) (-0.015 - 0.023)	-0.072, 0.075	0.964	0.90, 1.14 (0.85 - 1.23)
Histidine	0.76 (0.013) (0.73 - 0.79)	0.76 (0.015) (0.74 - 0.79)	0 (0.019) (-0.032 - 0.052)	-0.049, 0.049	0.999	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.96 (0.018) (0.90 - 1.00)	0.96 (0.020) (0.93 - 0.97)	-0.00058 (0.020) (-0.030 - 0.040)	-0.053, 0.051	0.978	0.75, 0.96 (0.72 - 1.03)

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.60 (0.023) (1.55 - 1.65)	1.60 (0.027) (1.55 - 1.63)	-0.0061 (0.035) (-0.025 - 0.023)	-0.097, 0.085	0.869	1.25, 1.62 (1.20 - 1.72)
Lysine	1.25 (0.029) (1.17 - 1.36)	1.26 (0.033) (1.22 - 1.29)	-0.010 (0.043) (-0.060 - 0.077)	-0.12, 0.10	0.819	1.01, 1.30 (0.99 - 1.44)
Methionine	0.40 (0.0093) (0.38 - 0.42)	0.41 (0.011) (0.40 - 0.42)	-0.011 (0.014) (-0.046 - 0.016)	-0.048, 0.026	0.477	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.48 (0.027) (1.44 - 1.54)	1.52 (0.031) (1.45 - 1.56)	-0.043 (0.041) (-0.058 - -0.012)	-0.15, 0.063	0.342	1.12, 1.58 (1.10 - 1.63)
Proline	1.03 (0.022) (0.98 - 1.11)	1.09 (0.025) (1.07 - 1.12)	-0.065 (0.029) (-0.094 - -0.010)	-0.14, 0.0098	0.075	0.83, 1.08 (0.79 - 1.17)
Serine	1.11 (0.027) (1.11 - 1.13)	1.13 (0.032) (1.09 - 1.19)	-0.017 (0.042) (-0.087 - 0.028)	-0.12, 0.090	0.696	0.83, 1.21 (0.81 - 1.24)

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.90 (0.014) (0.88 - 0.92)	0.89 (0.016) (0.86 - 0.92)	0.0096 (0.021) (-0.0050 - 0.015)	-0.045, 0.064	0.669	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.021) (0.40 - 0.44)	0.45 (0.024) (0.39 - 0.52)	-0.039 (0.032) (-0.081 - 0.024)	-0.12, 0.045	0.285	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.84 (0.012) (0.81 - 0.87)	0.84 (0.014) (0.82 - 0.87)	0.0015 (0.018) (-0.026 - 0.039)	-0.045, 0.047	0.937	0.67, 0.84 (0.63 - 0.91)
Valine	1.26 (0.025) (1.17 - 1.31)	1.30 (0.027) (1.24 - 1.32)	-0.038 (0.021) (-0.067 - -0.010)	-0.091, 0.016	0.130	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.68 (0.0074) (0.66 - 0.70)	0.75 (0.0086) (0.74 - 0.76)	-0.063 (0.011) (-0.077 - -0.044)	-0.091, -0.034	0.002	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	22.89 (0.12) (22.47 - 23.15)	23.10 (0.14) (23.07 - 23.15)	-0.21 (0.18) (-0.68 - 0.079)	-0.68, 0.25	0.286	16.54, 30.55 (19.11 - 26.73)

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.46 (0.0047) (0.44 - 0.47)	0.48 (0.0053) (0.47 - 0.49)	-0.018 (0.0055) (-0.025 - -0.0092)	-0.033, -0.0044	0.019	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.50 (0.037) (2.39 - 2.64)	2.34 (0.043) (2.32 - 2.38)	0.16 (0.057) (0.011 - 0.22)	0.015, 0.31	0.036	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	15.04 (0.12) (14.58 - 15.26)	14.70 (0.14) (14.51 - 14.83)	0.35 (0.19) (-0.17 - 0.75)	-0.14, 0.84	0.127	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	56.95 (0.23) (56.35 - 57.88)	57.19 (0.26) (57.01 - 57.46)	-0.24 (0.35) (-1.12 - 0.80)	-1.13, 0.66	0.528	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.31 (0.012) (0.27 - 0.34)	0.29 (0.014) (0.27 - 0.30)	0.028 (0.018) (-0.0012 - 0.052)	-0.018, 0.074	0.178	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.28 (0.0065) (0.26 - 0.30)	0.28 (0.0075) (0.27 - 0.28)	0.0046 (0.0099) (-0.024 - 0.024)	-0.021, 0.030	0.663	0.17, 0.38 (0.20 - 0.36)

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.16 (0.0056) (0.15 - 0.19)	0.15 (0.0065) (0.15 - 0.16)	0.011 (0.0086) (-0.0044 - 0.032)	-0.011, 0.033	0.258	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0014) (0.14 - 0.15)	0.14 (0.0017) (0.14 - 0.14)	0.0095 (0.0022) (0.0056 - 0.013)	0.0039, 0.015	0.007	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	6.82 (0.36) (5.81 - 7.58)	6.91 (0.41) (6.64 - 7.19)	-0.084 (0.54) (-1.38 - 0.53)	-1.48, 1.31	0.883	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	43.21 (1.00) (41.96 - 44.44)	48.04 (1.15) (45.03 - 50.87)	-4.83 (1.53) (-6.43 - -2.22)	-8.75, -0.90	0.025	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.41 (0.010) (0.40 - 0.43)	0.40 (0.012) (0.37 - 0.44)	0.011 (0.016) (-0.036 - 0.049)	-0.030, 0.052	0.529	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	14.12 (0.36) (13.57 - 14.81)	13.83 (0.42) (13.65 - 14.11)	0.29 (0.56) (-0.54 - 0.52)	-1.14, 1.72	0.622	9.07, 17.33 (9.07 - 17.14)

Table E-11. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.64 (0.020) (0.61 - 0.67)	0.66 (0.023) (0.59 - 0.71)	-0.020 (0.031) (-0.087 - 0.068)	-0.099, 0.059	0.548	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.06 (0.019) (1.04 - 1.09)	1.08 (0.022) (1.03 - 1.16)	-0.022 (0.029) (-0.12 - 0.032)	-0.097, 0.052	0.471	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.11 (0.0084) (0.068 - 0.12)	0.099 (0.0096) (0.094 - 0.10)	0.0074 (0.011) (-0.031 - 0.030)	-0.022, 0.036	0.539	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	40.79 (1.30) (40.28 - 41.37)	49.54 (1.50) (44.04 - 52.95)	-8.75 (1.98) (-11.57 - -3.76)	-13.84, -3.66	0.006	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	169.03 (3.66) (163.57 - 179.34)	156.99 (4.23) (151.55 - 162.98)	12.04 (5.60) (8.84 - 16.38)	-2.34, 26.43	0.084	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-12. Statistical Summary of Site NCBD Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.14 (0.0093) (0.13 - 0.15)	0.13 (0.011) (0.12 - 0.14)	0.012 (0.014) (0.0062 - 0.024)	-0.024, 0.048	0.422	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.36 (0.040) (0.20 - 0.43)	0.37 (0.046) (0.36 - 0.41)	-0.017 (0.056) (0.0058 - 0.056)	-0.16, 0.13	0.773	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.22 (0.024) (0.13 - 0.27)	0.22 (0.028) (0.21 - 0.23)	-0.0024 (0.037) (0.0078 - 0.040)	-0.098, 0.093	0.951	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.94 (0.026) (0.91 - 0.97)	0.90 (0.030) (0.81 - 0.95)	0.038 (0.039) (-0.024 - 0.097)	-0.063, 0.14	0.374	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.12 (0.067) (1.07 - 1.18)	1.09 (0.077) (1.08 - 1.10)	0.037 (0.10) (-0.0038 - 0.050)	-0.22, 0.30	0.731	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.16 (0.087) (3.77 - 4.38)	4.27 (0.10) (4.20 - 4.39)	-0.11 (0.13) (-0.093 - 0.13)	-0.45, 0.22	0.434	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	496.46 (2.64) (486.87 - 500.48)	492.81 (3.05) (490.52 - 494.31)	3.66 (4.04) (-7.44 - 9.96)	-6.73, 14.04	0.406	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	42.09 (0.46) (41.40 - 43.69)	42.60 (0.53) (42.14 - 43.05)	-0.50 (0.70) (-1.65 - 1.09)	-2.31, 1.30	0.504	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.59 (0.25) (5.93 - 7.28)	7.28 (0.28) (6.63 - 7.75)	-0.70 (0.32) (-1.82 - -0.34)	-1.53, 0.13	0.082	4.79, 9.92 (6.05 - 10.50)
Protein	31.15 (0.13) (30.63 - 31.47)	31.18 (0.15) (31.00 - 31.27)	-0.025 (0.19) (-0.65 - 0.46)	-0.52, 0.47	0.902	22.30, 29.41 (20.58 - 29.28)
Total Fat	22.59 (0.54) (20.62 - 23.58)	21.95 (0.62) (21.42 - 22.22)	0.64 (0.83) (-1.60 - 2.16)	-1.48, 2.77	0.471	15.01, 28.51 (16.58 - 25.25)

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	24.46 (0.51) (23.26 - 25.45)	26.40 (0.59) (25.76 - 27.10)	-1.94 (0.79) (-3.08 - -1.80)	-3.96, 0.080	0.056	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.90 (0.74) (17.33 - 18.57)	17.71 (0.84) (16.06 - 20.78)	0.20 (0.94) (-2.21 - 1.44)	-2.23, 2.63	0.841	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	29.73 (0.87) (27.53 - 32.00)	32.83 (1.00) (31.58 - 34.49)	-3.09 (1.32) (-6.95 - -0.41)	-6.49, 0.31	0.066	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.16 (0.71) (37.46 - 40.44)	41.10 (0.75) (39.09 - 43.00)	-1.94 (0.55) (-3.61 - -0.88)	-3.36, -0.53	0.016	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.11 (0.011) (1.10 - 1.13)	1.13 (0.013) (1.09 - 1.17)	-0.019 (0.014) (-0.044 - 0.0067)	-0.054, 0.015	0.212	0.86, 1.11 (0.83 - 1.22)
Arginine	3.48 (0.049) (3.42 - 3.60)	3.71 (0.053) (3.67 - 3.77)	-0.23 (0.048) (-0.34 - -0.17)	-0.35, -0.10	0.005	2.38, 3.47 (2.30 - 3.55)

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.60 (0.033) (2.55 - 2.64)	2.66 (0.037) (2.58 - 2.74)	-0.062 (0.043) (-0.11 - -0.031)	-0.17, 0.048	0.206	1.94, 2.57 (1.79 - 2.72)
Cystine	0.43 (0.013) (0.39 - 0.47)	0.44 (0.015) (0.43 - 0.45)	-0.010 (0.020) (-0.063 - 0.038)	-0.061, 0.040	0.620	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	5.30 (0.087) (5.24 - 5.38)	5.46 (0.10) (5.29 - 5.70)	-0.16 (0.13) (-0.46 - -0.0085)	-0.50, 0.18	0.285	3.74, 5.28 (3.39 - 5.45)
Glycine	1.16 (0.014) (1.14 - 1.19)	1.18 (0.015) (1.17 - 1.20)	-0.021 (0.016) (-0.037 - -0.013)	-0.063, 0.020	0.247	0.90, 1.14 (0.85 - 1.23)
Histidine	0.82 (0.010) (0.80 - 0.85)	0.83 (0.012) (0.83 - 0.84)	-0.0065 (0.015) (-0.026 - 0.024)	-0.044, 0.031	0.675	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.97 (0.017) (0.94 - 1.01)	0.98 (0.018) (0.94 - 1.03)	-0.011 (0.012) (-0.038 - 0.021)	-0.041, 0.019	0.397	0.75, 0.96 (0.72 - 1.03)

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.65 (0.021) (1.62 - 1.70)	1.66 (0.023) (1.63 - 1.69)	-0.0069 (0.022) (-0.039 - 0.0098)	-0.063, 0.049	0.763	1.25, 1.62 (1.20 - 1.72)
Lysine	1.33 (0.019) (1.26 - 1.38)	1.36 (0.022) (1.32 - 1.39)	-0.030 (0.022) (-0.058 - -0.0056)	-0.087, 0.027	0.232	1.01, 1.30 (0.99 - 1.44)
Methionine	0.43 (0.012) (0.40 - 0.46)	0.40 (0.014) (0.38 - 0.41)	0.033 (0.018) (-0.013 - 0.071)	-0.014, 0.081	0.129	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.61 (0.026) (1.56 - 1.66)	1.61 (0.028) (1.60 - 1.66)	-0.0044 (0.022) (-0.038 - 0.015)	-0.061, 0.052	0.850	1.12, 1.58 (1.10 - 1.63)
Proline	1.14 (0.027) (1.09 - 1.21)	1.18 (0.031) (1.10 - 1.25)	-0.040 (0.039) (-0.080 - -0.015)	-0.14, 0.060	0.353	0.83, 1.08 (0.79 - 1.17)
Serine	1.17 (0.026) (1.15 - 1.23)	1.20 (0.030) (1.16 - 1.24)	-0.028 (0.040) (-0.069 - -0.0042)	-0.13, 0.075	0.512	0.83, 1.21 (0.81 - 1.24)

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.92 (0.015) (0.89 - 0.94)	0.93 (0.017) (0.91 - 0.94)	-0.0072 (0.021) (-0.024 - 0.0027)	-0.062, 0.048	0.747	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.46 (0.014) (0.43 - 0.52)	0.44 (0.016) (0.43 - 0.45)	0.019 (0.020) (-0.019 - 0.076)	-0.032, 0.071	0.382	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.87 (0.014) (0.85 - 0.92)	0.89 (0.015) (0.86 - 0.91)	-0.012 (0.014) (-0.039 - 0.0089)	-0.048, 0.024	0.440	0.67, 0.84 (0.63 - 0.91)
Valine	1.32 (0.024) (1.26 - 1.40)	1.34 (0.026) (1.31 - 1.40)	-0.021 (0.019) (-0.048 - 0.0048)	-0.070, 0.029	0.329	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.93 (0.0046) (0.92 - 0.95)	0.98 (0.0054) (0.97 - 0.98)	-0.043 (0.0071) (-0.060 - -0.037)	-0.062, -0.025	0.001	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.19 (0.088) (24.02 - 24.42)	24.11 (0.10) (23.89 - 24.34)	0.083 (0.13) (-0.32 - 0.33)	-0.26, 0.43	0.562	16.54, 30.55 (19.11 - 26.73)

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.53 (0.0022) (0.52 - 0.53)	0.54 (0.0025) (0.53 - 0.54)	-0.012 (0.0033) (-0.019 - -0.0082)	-0.021, -0.0037	0.014	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.51 (0.020) (2.47 - 2.56)	2.64 (0.021) (2.61 - 2.70)	-0.14 (0.016) (-0.15 - -0.095)	-0.18, -0.094	<0.001	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	16.21 (0.067) (16.03 - 16.40)	16.21 (0.076) (16.10 - 16.35)	0.0024 (0.088) (-0.11 - 0.24)	-0.22, 0.23	0.979	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	54.32 (0.084) (54.30 - 54.33)	54.29 (0.097) (54.04 - 54.50)	0.029 (0.13) (-0.18 - 0.30)	-0.30, 0.36	0.833	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.16 (0.0019) (0.15 - 0.16)	0.14 (0.0022) (0.14 - 0.15)	0.012 (0.0029) (0.0078 - 0.014)	0.0043, 0.019	0.009	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.30 (0.0052) (0.29 - 0.30)	0.31 (0.0060) (0.28 - 0.32)	-0.011 (0.0080) (-0.025 - 0.014)	-0.031, 0.0095	0.225	0.17, 0.38 (0.20 - 0.36)

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.16 (0.0065) (0.16 - 0.17)	0.19 (0.0075) (0.17 - 0.21)	-0.023 (0.0099) (-0.049 - -0.0086)	-0.049, 0.0021	0.065	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0029) (0.14 - 0.15)	0.13 (0.0034) (0.12 - 0.13)	0.021 (0.0042) (0.0081 - 0.031)	0.011, 0.032	0.003	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	11.35 (0.15) (11.11 - 11.91)	11.75 (0.17) (11.46 - 11.92)	-0.40 (0.22) (-0.76 - 0.060)	-0.97, 0.18	0.134	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	63.88 (3.33) (60.27 - 66.59)	64.62 (3.84) (63.58 - 66.45)	-0.74 (5.08) (-6.18 - 2.76)	-13.80, 12.32	0.890	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.39 (0.0053) (0.38 - 0.41)	0.37 (0.0061) (0.36 - 0.38)	0.019 (0.0081) (0.0045 - 0.036)	-0.0015, 0.040	0.062	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	12.93 (0.23) (12.73 - 13.13)	12.90 (0.26) (12.00 - 13.47)	0.029 (0.34) (-0.47 - 1.13)	-0.86, 0.92	0.936	9.07, 17.33 (9.07 - 17.14)

Table E-13. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.77 (0.010) (0.74 - 0.80)	0.79 (0.012) (0.78 - 0.80)	-0.020 (0.016) (-0.035 - 0.019)	-0.060, 0.021	0.264	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.10 (0.016) (1.05 - 1.14)	1.11 (0.018) (1.07 - 1.14)	-0.0086 (0.017) (-0.020 - -0.0045)	-0.052, 0.035	0.636	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.021 (0.0034) (0.019 - 0.022)	0.013 (0.0039) (0.0054 - 0.023)	0.0075 (0.0052) (-0.0044 - 0.016)	-0.0057, 0.021	0.203	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	45.63 (0.60) (44.12 - 46.74)	49.43 (0.69) (47.66 - 50.87)	-3.80 (0.92) (-5.64 - -0.92)	-6.16, -1.44	0.009	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	114.29 (2.04) (107.78 - 119.15)	112.18 (2.36) (107.02 - 115.99)	2.11 (3.12) (-5.75 - 12.13)	-5.90, 10.12	0.528	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-14. Statistical Summary of Site NMLC Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.16 (0.0079) (0.14 - 0.18)	0.14 (0.0092) (0.12 - 0.15)	0.019 (0.012) (-0.0065 - 0.041)	-0.012, 0.050	0.178	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.34 (0.023) (0.30 - 0.38)	0.29 (0.026) (0.26 - 0.31)	0.054 (0.035) (-0.0093 - 0.074)	-0.035, 0.14	0.177	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.20 (0.018) (0.18 - 0.23)	0.18 (0.020) (0.17 - 0.19)	0.018 (0.027) (-0.012 - 0.032)	-0.051, 0.087	0.531	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.85 (0.026) (0.83 - 0.88)	0.69 (0.030) (0.68 - 0.70)	0.15 (0.040) (0.14 - 0.18)	0.052, 0.26	0.011	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.92 (0.026) (0.84 - 0.97)	0.80 (0.030) (0.74 - 0.87)	0.12 (0.040) (0.060 - 0.18)	0.022, 0.23	0.026	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.11 (0.095) (3.99 - 4.28)	3.74 (0.095) (3.38 - 3.98)	0.37 (0.10) (0.25 - 0.61)	0.12, 0.62	0.010	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	511.69 (2.44) (505.01 - 517.46)	503.38 (2.44) (499.09 - 512.65)	8.31 (3.45) (-7.65 - 18.37)	-0.13, 16.75	0.052	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	46.56 (0.54) (45.10 - 47.48)	48.67 (0.54) (47.50 - 49.59)	-2.11 (0.75) (-3.20 - -0.23)	-3.95, -0.27	0.031	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.73 (0.17) (6.27 - 7.13)	7.08 (0.17) (6.63 - 7.37)	-0.35 (0.19) (-0.89 - 0.030)	-0.81, 0.12	0.119	4.79, 9.92 (6.05 - 10.50)
Protein	23.70 (0.42) (22.71 - 24.70)	23.92 (0.42) (23.56 - 24.61)	-0.22 (0.49) (-0.85 - 0.64)	-1.43, 0.98	0.669	22.30, 29.41 (20.58 - 29.28)
Total Fat	25.65 (0.44) (24.23 - 26.78)	23.65 (0.44) (22.92 - 25.20)	2.00 (0.63) (-0.97 - 3.86)	0.46, 3.54	0.019	15.01, 28.51 (16.58 - 25.25)

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.95 (0.44) (24.75 - 26.52)	27.04 (0.44) (26.24 - 27.74)	-1.09 (0.60) (-1.75 - -0.099)	-2.56, 0.37	0.118	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	19.72 (0.80) (17.98 - 21.66)	19.13 (0.80) (16.91 - 21.70)	0.59 (1.13) (-2.59 - 4.75)	-2.17, 3.36	0.617	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	31.34 (0.67) (29.42 - 32.89)	33.60 (0.67) (32.74 - 35.52)	-2.27 (0.95) (-6.10 - -0.44)	-4.59, 0.049	0.053	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.72 (0.62) (38.66 - 40.44)	41.87 (0.62) (40.16 - 43.29)	-2.14 (0.88) (-3.56 - -0.63)	-4.29, 0.0015	0.050	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	0.96 (0.022) (0.91 - 1.00)	0.94 (0.022) (0.88 - 0.97)	0.017 (0.029) (-0.020 - 0.033)	-0.055, 0.089	0.583	0.86, 1.11 (0.83 - 1.22)
Arginine	2.52 (0.088) (2.33 - 2.74)	2.59 (0.088) (2.41 - 2.71)	-0.063 (0.10) (-0.18 - 0.021)	-0.31, 0.18	0.556	2.38, 3.47 (2.30 - 3.55)

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.08 (0.054) (1.94 - 2.20)	2.07 (0.054) (1.92 - 2.18)	0.0087 (0.070) (-0.085 - 0.096)	-0.16, 0.18	0.904	1.94, 2.57 (1.79 - 2.72)
Cystine	0.36 (0.017) (0.32 - 0.41)	0.35 (0.017) (0.31 - 0.39)	0.0082 (0.018) (-0.018 - 0.024)	-0.036, 0.052	0.666	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.14 (0.13) (3.80 - 4.40)	4.10 (0.13) (3.66 - 4.40)	0.039 (0.18) (-0.11 - 0.14)	-0.40, 0.47	0.833	3.74, 5.28 (3.39 - 5.45)
Glycine	0.99 (0.022) (0.93 - 1.04)	0.98 (0.022) (0.91 - 1.02)	0.016 (0.031) (-0.0040 - 0.033)	-0.061, 0.093	0.627	0.90, 1.14 (0.85 - 1.23)
Histidine	0.64 (0.021) (0.58 - 0.70)	0.64 (0.021) (0.61 - 0.66)	0.0012 (0.025) (-0.053 - 0.033)	-0.060, 0.062	0.961	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.81 (0.023) (0.75 - 0.88)	0.82 (0.023) (0.77 - 0.83)	-0.0053 (0.030) (-0.077 - 0.054)	-0.078, 0.068	0.865	0.75, 0.96 (0.72 - 1.03)

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.36 (0.034) (1.29 - 1.46)	1.36 (0.034) (1.28 - 1.40)	0.0086 (0.046) (-0.053 - 0.064)	-0.10, 0.12	0.858	1.25, 1.62 (1.20 - 1.72)
Lysine	1.12 (0.027) (1.05 - 1.18)	1.11 (0.027) (1.06 - 1.17)	0.0024 (0.025) (-0.018 - 0.026)	-0.059, 0.064	0.925	1.01, 1.30 (0.99 - 1.44)
Methionine	0.38 (0.016) (0.35 - 0.42)	0.33 (0.016) (0.32 - 0.35)	0.041 (0.020) (0.017 - 0.077)	-0.0093, 0.091	0.093	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.22 (0.033) (1.14 - 1.31)	1.23 (0.033) (1.15 - 1.27)	-0.0097 (0.039) (-0.075 - 0.043)	-0.11, 0.086	0.811	1.12, 1.58 (1.10 - 1.63)
Proline	0.87 (0.026) (0.82 - 0.92)	0.87 (0.026) (0.81 - 0.93)	0.0028 (0.026) (-0.035 - 0.034)	-0.060, 0.066	0.916	0.83, 1.08 (0.79 - 1.17)
Serine	0.98 (0.028) (0.90 - 1.04)	0.96 (0.028) (0.86 - 1.03)	0.019 (0.033) (-0.0099 - 0.042)	-0.062, 0.10	0.587	0.83, 1.21 (0.81 - 1.24)

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.79 (0.019) (0.74 - 0.83)	0.77 (0.019) (0.73 - 0.81)	0.016 (0.023) (0.0053 - 0.039)	-0.041, 0.073	0.518	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.35 (0.0093) (0.33 - 0.38)	0.38 (0.0093) (0.37 - 0.40)	-0.025 (0.0085) (-0.040 - 0.0010)	-0.046, -0.0048	0.023	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.72 (0.020) (0.67 - 0.78)	0.71 (0.020) (0.67 - 0.74)	0.0042 (0.025) (-0.033 - 0.040)	-0.058, 0.066	0.873	0.67, 0.84 (0.63 - 0.91)
Valine	1.07 (0.029) (1.00 - 1.14)	1.07 (0.029) (1.00 - 1.10)	0.0056 (0.039) (-0.084 - 0.049)	-0.090, 0.10	0.889	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.70 (0.011) (0.67 - 0.72)	0.73 (0.011) (0.72 - 0.75)	-0.028 (0.012) (-0.049 - 0.0062)	-0.057, 0.00046	0.052	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.74 (0.086) (24.59 - 24.94)	24.39 (0.086) (24.07 - 24.59)	0.35 (0.12) (0.17 - 0.61)	0.049, 0.65	0.029	16.54, 30.55 (19.11 - 26.73)

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.48 (0.0056) (0.47 - 0.49)	0.48 (0.0056) (0.47 - 0.49)	-0.0075 (0.0076) (-0.020 - 0.018)	-0.026, 0.011	0.361	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.78 (0.036) (2.75 - 2.85)	2.67 (0.036) (2.58 - 2.76)	0.11 (0.050) (0.0076 - 0.18)	-0.015, 0.23	0.076	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.40 (0.11) (14.15 - 14.68)	14.46 (0.11) (14.42 - 14.49)	-0.059 (0.15) (-0.33 - 0.19)	-0.43, 0.31	0.706	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.54 (0.18) (55.18 - 55.96)	55.87 (0.18) (55.61 - 56.29)	-0.33 (0.25) (-1.11 - 0.13)	-0.94, 0.29	0.242	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.15 (0.0017) (0.15 - 0.16)	0.15 (0.0017) (0.14 - 0.15)	0.0044 (0.0023) (-0.00022 - 0.011)	-0.0012, 0.0099	0.103	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.29 (0.0070) (0.27 - 0.31)	0.30 (0.0070) (0.29 - 0.30)	-0.0046 (0.0099) (-0.027 - 0.019)	-0.029, 0.020	0.656	0.17, 0.38 (0.20 - 0.36)

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.14 (0.0033) (0.13 - 0.14)	0.14 (0.0033) (0.13 - 0.14)	-0.0014 (0.0046) (-0.0030 - 0.00006)	-0.013, 0.0099	0.765	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.11 (0.0040) (0.10 - 0.11)	0.091 (0.0040) (0.081 - 0.095)	0.016 (0.0056) (0.0059 - 0.023)	0.0025, 0.030	0.027	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	5.82 (0.24) (5.22 - 6.30)	5.64 (0.24) (5.40 - 5.85)	0.18 (0.32) (-0.28 - 0.55)	-0.61, 0.97	0.593	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	63.78 (4.68) (59.75 - 67.62)	73.46 (4.68) (63.01 - 89.93)	-9.68 (4.30) (-22.31 - -1.16)	-20.21, 0.84	0.065	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.39 (0.0090) (0.37 - 0.41)	0.36 (0.0090) (0.34 - 0.37)	0.033 (0.0080) (0.014 - 0.044)	0.014, 0.053	0.005	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	11.39 (0.51) (10.88 - 11.68)	9.72 (0.51) (8.61 - 11.03)	1.67 (0.72) (0.65 - 2.27)	-0.080, 3.42	0.058	9.07, 17.33 (9.07 - 17.14)

Table E-15. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.67 (0.022) (0.64 - 0.71)	0.63 (0.022) (0.58 - 0.68)	0.035 (0.018) (0.0036 - 0.072)	-0.0093, 0.080	0.100	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.13 (0.031) (1.11 - 1.17)	1.02 (0.031) (0.88 - 1.08)	0.11 (0.043) (0.046 - 0.23)	0.0051, 0.22	0.042	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.022 (0.0033) (0.018 - 0.027)	0.015 (0.0033) (0.012 - 0.023)	0.0070 (0.0046) (0.0039 - 0.013)	-0.0043, 0.018	0.180	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	29.14 (0.82) (27.31 - 31.57)	30.08 (0.82) (28.22 - 31.74)	-0.94 (0.82) (-2.85 - 1.07)	-2.96, 1.08	0.297	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	162.17 (1.77) (158.92 - 165.82)	158.20 (1.77) (153.15 - 162.63)	3.97 (2.50) (2.38 - 7.55)	-2.16, 10.10	0.164	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-16. Statistical Summary of Site SCEK Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0087) (0.12 - 0.18)	0.15 (0.0087) (0.14 - 0.15)	0.0014 (0.012) (-0.023 - 0.043)	-0.029, 0.031	0.914	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.41 (0.029) (0.33 - 0.52)	0.43 (0.029) (0.39 - 0.46)	-0.022 (0.041) (-0.11 - 0.13)	-0.12, 0.078	0.607	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.014) (0.19 - 0.28)	0.24 (0.014) (0.22 - 0.25)	-0.011 (0.020) (-0.060 - 0.060)	-0.060, 0.038	0.607	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	1.10 (0.029) (1.05 - 1.18)	1.13 (0.029) (1.06 - 1.20)	-0.030 (0.027) (-0.086 - 0.00085)	-0.096, 0.035	0.303	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.17 (0.025) (1.13 - 1.23)	1.07 (0.025) (1.05 - 1.10)	0.10 (0.031) (0.074 - 0.13)	0.026, 0.18	0.017	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	3.85 (0.051) (3.77 - 3.92)	3.46 (0.051) (3.34 - 3.61)	0.40 (0.072) (0.24 - 0.58)	0.22, 0.57	0.001	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	498.01 (2.54) (489.04 - 502.78)	494.42 (2.54) (489.10 - 500.98)	3.59 (3.47) (-1.24 - 13.67)	-4.90, 12.07	0.340	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.03 (0.48) (42.73 - 45.99)	46.39 (0.48) (45.65 - 47.07)	-2.36 (0.64) (-3.73 - -0.88)	-3.92, -0.79	0.010	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.88 (0.21) (6.32 - 7.37)	7.47 (0.21) (7.11 - 7.79)	-0.59 (0.29) (-1.47 - 0.18)	-1.30, 0.13	0.090	4.79, 9.92 (6.05 - 10.50)
Protein	29.43 (0.24) (29.06 - 30.14)	28.48 (0.24) (28.09 - 28.77)	0.95 (0.29) (0.38 - 1.82)	0.24, 1.66	0.017	22.30, 29.41 (20.58 - 29.28)
Total Fat	22.71 (0.48) (20.94 - 23.59)	21.70 (0.48) (20.71 - 22.88)	1.01 (0.65) (0.15 - 2.88)	-0.58, 2.61	0.169	15.01, 28.51 (16.58 - 25.25)

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.53 (0.37) (25.31 - 25.83)	25.53 (0.37) (24.51 - 26.91)	0.0023 (0.41) (-1.08 - 0.91)	-1.01, 1.01	0.995	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.93 (0.38) (17.17 - 18.84)	18.10 (0.38) (17.35 - 19.63)	-0.17 (0.45) (-1.48 - 1.07)	-1.26, 0.92	0.716	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	29.75 (0.41) (28.74 - 30.56)	32.12 (0.41) (30.49 - 33.05)	-2.38 (0.58) (-4.31 - -0.26)	-3.80, -0.96	0.006	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.54 (0.62) (38.76 - 40.86)	40.47 (0.62) (39.15 - 42.09)	-0.93 (0.72) (-3.24 - 0.56)	-2.69, 0.83	0.245	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.07 (0.019) (1.05 - 1.10)	1.05 (0.019) (0.97 - 1.10)	0.022 (0.026) (-0.040 - 0.12)	-0.042, 0.086	0.438	0.86, 1.11 (0.83 - 1.22)
Arginine	3.25 (0.074) (3.15 - 3.33)	3.25 (0.074) (2.94 - 3.49)	-0.0020 (0.10) (-0.34 - 0.39)	-0.26, 0.25	0.985	2.38, 3.47 (2.30 - 3.55)

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.51 (0.047) (2.43 - 2.55)	2.45 (0.047) (2.26 - 2.62)	0.060 (0.067) (-0.092 - 0.29)	-0.10, 0.22	0.407	1.94, 2.57 (1.79 - 2.72)
Cystine	0.41 (0.015) (0.39 - 0.43)	0.40 (0.015) (0.36 - 0.45)	0.0068 (0.017) (-0.030 - 0.073)	-0.034, 0.047	0.697	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.94 (0.13) (4.73 - 5.14)	5.02 (0.13) (4.41 - 5.32)	-0.072 (0.19) (-0.44 - 0.73)	-0.53, 0.39	0.714	3.74, 5.28 (3.39 - 5.45)
Glycine	1.12 (0.022) (1.07 - 1.15)	1.11 (0.022) (1.03 - 1.19)	0.0062 (0.031) (-0.073 - 0.12)	-0.070, 0.082	0.849	0.90, 1.14 (0.85 - 1.23)
Histidine	0.77 (0.018) (0.73 - 0.81)	0.76 (0.018) (0.71 - 0.82)	0.017 (0.026) (-0.062 - 0.091)	-0.046, 0.079	0.538	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.95 (0.013) (0.93 - 0.97)	0.93 (0.013) (0.89 - 0.97)	0.013 (0.017) (-0.023 - 0.051)	-0.027, 0.054	0.457	0.75, 0.96 (0.72 - 1.03)

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control)			Commercial Tolerance Interval[5] (Range)
			Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	
Amino Acid (% dw)						
Leucine	1.58 (0.026) (1.52 - 1.61)	1.55 (0.026) (1.45 - 1.64)	0.028 (0.037) (-0.072 - 0.16)	-0.064, 0.12	0.486	1.25, 1.62 (1.20 - 1.72)
Lysine	1.26 (0.026) (1.21 - 1.33)	1.24 (0.026) (1.19 - 1.33)	0.024 (0.037) (-0.11 - 0.13)	-0.067, 0.12	0.537	1.01, 1.30 (0.99 - 1.44)
Methionine	0.40 (0.021) (0.37 - 0.44)	0.39 (0.021) (0.32 - 0.44)	0.016 (0.029) (-0.066 - 0.12)	-0.056, 0.088	0.605	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.51 (0.034) (1.46 - 1.55)	1.48 (0.034) (1.36 - 1.61)	0.026 (0.048) (-0.14 - 0.19)	-0.092, 0.14	0.614	1.12, 1.58 (1.10 - 1.63)
Proline	1.04 (0.024) (0.99 - 1.11)	1.04 (0.024) (1.01 - 1.11)	0.0028 (0.033) (-0.12 - 0.10)	-0.079, 0.084	0.935	0.83, 1.08 (0.79 - 1.17)
Serine	1.11 (0.031) (1.06 - 1.13)	1.11 (0.031) (0.97 - 1.17)	0.00043 (0.044) (-0.087 - 0.16)	-0.11, 0.11	0.992	0.83, 1.21 (0.81 - 1.24)

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control)			Commercial Tolerance Interval[5] (Range)
			Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	
Amino Acid (% dw)						
Threonine	0.89 (0.016) (0.86 - 0.92)	0.86 (0.016) (0.80 - 0.91)	0.032 (0.022) (-0.026 - 0.10)	-0.022, 0.086	0.197	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.42 (0.013) (0.40 - 0.44)	0.43 (0.013) (0.38 - 0.47)	-0.0094 (0.018) (-0.070 - 0.030)	-0.054, 0.035	0.626	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.84 (0.017) (0.82 - 0.86)	0.81 (0.017) (0.74 - 0.87)	0.031 (0.024) (-0.048 - 0.12)	-0.028, 0.089	0.245	0.67, 0.84 (0.63 - 0.91)
Valine	1.26 (0.030) (1.21 - 1.29)	1.23 (0.030) (1.16 - 1.29)	0.024 (0.042) (-0.046 - 0.12)	-0.079, 0.13	0.586	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.86 (0.010) (0.84 - 0.88)	0.84 (0.010) (0.81 - 0.87)	0.017 (0.013) (-0.010 - 0.047)	-0.016, 0.050	0.246	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	23.15 (0.14) (22.72 - 23.62)	23.01 (0.14) (22.86 - 23.25)	0.13 (0.16) (-0.27 - 0.76)	-0.27, 0.53	0.443	16.54, 30.55 (19.11 - 26.73)

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control)			Commercial Tolerance Interval[5] (Range)
			Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.48 (0.0058) (0.47 - 0.49)	0.46 (0.0058) (0.45 - 0.47)	0.016 (0.0072) (0.0040 - 0.039)	-0.0018, 0.034	0.070	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.35 (0.025) (2.30 - 2.43)	2.46 (0.025) (2.40 - 2.52)	-0.11 (0.028) (-0.16 - -0.097)	-0.18, -0.047	0.006	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	16.34 (0.078) (16.22 - 16.45)	16.16 (0.078) (15.86 - 16.44)	0.18 (0.11) (-0.14 - 0.53)	-0.083, 0.45	0.143	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.42 (0.21) (54.97 - 55.95)	55.58 (0.21) (55.18 - 56.13)	-0.15 (0.29) (-1.16 - 0.77)	-0.87, 0.56	0.616	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.17 (0.0056) (0.17 - 0.18)	0.17 (0.0056) (0.16 - 0.17)	0.0057 (0.0079) (0.00091 - 0.011)	-0.014, 0.025	0.497	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.28 (0.0055) (0.27 - 0.28)	0.28 (0.0055) (0.28 - 0.29)	-0.0041 (0.0078) (-0.0061 - -0.0022)	-0.023, 0.015	0.618	0.17, 0.38 (0.20 - 0.36)

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.16 (0.0015) (0.16 - 0.16)	0.16 (0.0015) (0.16 - 0.16)	0.0020 (0.0020) (-0.00054 - 0.0042)	-0.0029, 0.0068	0.362	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.16 (0.0016) (0.16 - 0.16)	0.14 (0.0016) (0.13 - 0.14)	0.021 (0.0023) (0.018 - 0.023)	0.015, 0.027	<0.001	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	10.49 (0.15) (10.09 - 10.87)	9.98 (0.15) (9.58 - 10.41)	0.51 (0.22) (0.12 - 1.29)	-0.023, 1.04	0.057	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	60.47 (5.67) (56.94 - 66.50)	79.02 (5.67) (67.45 - 95.10)	-18.55 (7.06) (-38.15 - -0.95)	-35.82, -1.28	0.039	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.35 (0.0045) (0.35 - 0.37)	0.34 (0.0045) (0.33 - 0.34)	0.019 (0.0040) (0.0082 - 0.024)	0.0087, 0.028	0.003	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	10.91 (0.34) (10.18 - 11.37)	9.04 (0.34) (8.83 - 9.54)	1.86 (0.48) (0.64 - 2.54)	0.70, 3.03	0.007	9.07, 17.33 (9.07 - 17.14)

Table E-17. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control)			Commercial Tolerance Interval[5] (Range)
			Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	
Mineral						
Phosphorus (% dw)	0.58 (0.0099) (0.56 - 0.61)	0.57 (0.0099) (0.54 - 0.60)	0.0036 (0.011) (-0.0098 - 0.018)	-0.022, 0.030	0.742	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.01 (0.023) (0.98 - 1.06)	0.87 (0.023) (0.79 - 0.93)	0.14 (0.033) (0.073 - 0.27)	0.062, 0.22	0.004	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.024 (0.010) (0.019 - 0.027)	0.047 (0.010) (0.019 - 0.090)	-0.023 (0.014) (-0.065 - 0.0062)	-0.058, 0.012	0.161	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	34.10 (0.45) (33.36 - 35.30)	34.96 (0.45) (33.70 - 35.89)	-0.86 (0.64) (-2.31 - 1.61)	-2.44, 0.71	0.227	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	114.39 (2.75) (107.81 - 118.39)	103.66 (2.75) (93.92 - 109.90)	10.73 (3.90) (6.69 - 14.40)	1.20, 20.26	0.033	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-18. Statistical Summary of Site TXPL Cottonseed Anti-nutrients for MON 88701 (Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.16 (0.0047) (0.14 - 0.17)	0.16 (0.0047) (0.15 - 0.17)	-0.0044 (0.0066) (-0.026 - 0.021)	-0.021, 0.012	0.533	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.41 (0.020) (0.33 - 0.47)	0.47 (0.020) (0.44 - 0.49)	-0.054 (0.029) (-0.16 - 0.0068)	-0.12, 0.017	0.112	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.013) (0.18 - 0.27)	0.26 (0.013) (0.25 - 0.27)	-0.026 (0.018) (-0.085 - 0.024)	-0.070, 0.017	0.189	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.98 (0.024) (0.91 - 1.06)	0.93 (0.024) (0.91 - 0.95)	0.054 (0.033) (-0.025 - 0.13)	-0.028, 0.14	0.157	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.06 (0.024) (1.03 - 1.11)	1.01 (0.024) (0.97 - 1.05)	0.053 (0.022) (-0.021 - 0.092)	-0.00013, 0.11	0.050	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]MON 88701 plants were treated with dicamba and glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

E.5. Composition of Cottonseed Not Treated with Dicamba or Glufosinate

E.5.1. Nutrient Levels in Cottonseed Not Treated with Dicamba or Glufosinate

In the combined-site analysis of nutrient levels in cottonseed, the following components had no significant differences ($p<0.05$) in mean values between MON 88701 not treated with dicamba or glufosinate and the conventional control: one proximate (protein), 17 amino acids (alanine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine), eight fatty acids (16:0 palmitic acid, 16:1 palmitoleic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, and 22:0 behenic acid), and four minerals (copper, iron, phosphorus, and sodium) (Tables E-19 and E-20).

The nutrient components that had significant differences in mean values between MON 88701 and the conventional control in the combined-site analysis were: five proximates (ash, calories, carbohydrates, moisture, and total fat), four types of fiber (ADF, crude fiber, NDF, and TDF), one amino acid (arginine), one fatty acid (14:0 myristic acid), five minerals (calcium, magnesium, manganese, potassium, and zinc) and vitamin E (Table E-19).

The significant differences in nutrients were further evaluated using considerations relevant to the safety and nutritional quality of MON 88701 when compared to the conventional control:

All nutrient component differences observed in the combined-site statistical analysis, whether reflecting increased or decreased MON 88701 mean values, with respect to the conventional control, were 16.14% or less. The relative magnitude of the differences were as follows: 1.04 to 6.45% for proximates; 3.35 to 4.12% for fibers; 3.82% for arginine; 2.39% for 14:0 myristic acid; 5.48 to 16.14% for minerals; and 5.84% for vitamin E.

1) All mean values for all significantly different nutrient components from the combined-site analysis of MON 88701 were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial.

2) Assessment of the reproducibility of the combined-site differences at the eight individual sites showed statistically significant differences for: calories, carbohydrates, total fat, crude fiber, and NDF at one site; moisture, ADF, TDF, arginine and zinc at two sites; 14:0 myristic, potassium, and vitamin E at three sites; ash and magnesium at four sites, manganese at five sites and calcium at 7 sites. With the exception of potassium, arginine and zinc, each at a single site, all individual site mean values of MON 88701 for all nutrient components with significant differences were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial. The control values for arginine and zinc were also outside the tolerance interval at the individual sites where the MON 88701 value was outside the tolerance interval.

3) With the exception of calories, combined-site mean values and individual site mean values of MON 88701 for all nutrient components including those that were significantly different were within the context of the natural variability of commercial cotton

composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Five of the 17 cottonseed nutrient statistically significant differences between MON 88701 and the conventional control observed in the combined-site data analysis were attributable to small differences in proximates (ash, carbohydrates expressed as % dw, calories expressed as Kcal/100g % dw, moisture expressed as % fw and total fat expressed as % dw). For ash, calories, and total fat, the relative magnitudes of the differences between the mean values for MON 88701 and the conventional control were all small increases (4.77% for ash, 1.04% for calories and 5.37% for total fat). The relative magnitudes of differences for mean values of carbohydrates and moisture between MON 88701 and the conventional control were both small decreases; 2.96% for carbohydrates and 6.45% for moisture. All of the nutrient mean values for MON 88701 observed in the combined-site analysis for proximates were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for proximate mean values between MON 88701 and the conventional control were not consistently observed among sites. Ash was significantly different at four individual sites, with relative magnitudes of differences ranging from 4.34 to 11.0%. Moisture was decreased at two sites, with relative magnitude of differences ranging from 8.51 to 11.58%, carbohydrates had a relative decrease of 4.71% at one site. Both calories and total fat had small increases at one site with 1.30% for calories and 6.90% for total fat. Overall, observed differences in proximate values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were small, not consistently reproduced across the individual sites, and the mean MON 88701 combined-site values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Four of the 17 cottonseed nutrient differences between MON 88701 and the conventional control observed in the combined-site data analysis were attributable to small differences in fiber (ADF, crude fiber, NDF, and TDF, all expressed as % dw). All relative magnitudes of the differences between the mean value for MON 88701 and the conventional control were small decreases (3.93% for ADF, 4.12% for crude fiber, 3.56% for NDF, and 3.35% for TDF). All of the nutrient mean values for MON 88701 observed in the combined-site analysis for fiber were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for fiber mean values between MON 88701 and the conventional control were not consistently observed among individual sites. ADF and TDF were significantly different at two sites, with small decreases ranging from 8.50 to 8.91% for ADF and 5.21 to 6.83% for TDF. Crude fiber and NDF were significantly different at one site, with a small decrease of 5.46% for crude fiber and 5.66% for NDF. Overall, observed differences in fiber values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were small, not consistently reproduced across the individual sites, and the mean MON 88701 combined-site values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and within the context of the

natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

One of the cottonseed nutrient differences between MON 88701 and the conventional control observed in the combined-site analysis was attributed to a small difference in one amino acid (arginine, expressed as % dw). The relative magnitude of the difference between the mean value for MON 88701 and the conventional control was a small decrease of 3.82%. The mean arginine value for MON 88701 observed in the combined-site analysis was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for arginine mean values between MON 88701 and the conventional control were not consistently observed among individual sites, with small decreases ranging from 5.36 to 8.48% observed at two sites. Overall, observed differences in arginine values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were small, not consistently reproduced across the individual sites, and the mean MON 88701 combined-site values was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial or within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

One of the cottonseed nutrient differences between MON 88701 and the conventional control observed in the combined-site analysis was attributed to the fatty acid 14:0 myristic acid (expressed as % total FA). The relative magnitude of the difference between the mean fatty acid value for MON 88701 and the conventional control in the combined-site analysis was a small decrease of 2.39%. The mean 14:0 myristic acid value for MON 88701 observed in the combined-site analysis was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for 14:0 myristic acid mean values between MON 88701 and the conventional control were not consistently observed among individual sites, with three sites with small decreases ranging from 3.08 to 4.79%. Overall, observed differences in 14:0 myristic acid values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety and nutritional perspective because they were small, not consistently reproduced across the individual sites, and the mean MON 88701 combined-site value was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

Five of the 17 cottonseed nutrient differences between MON 88701 and the conventional control observed in the combined-site data analysis were attributable to small differences in minerals (calcium, magnesium, and potassium expressed as % dw and manganese and zinc expressed as mg/kg dw). For calcium, magnesium, manganese and potassium, the relative magnitude of the differences between the mean values for MON 88701 and the conventional control were all increases (14.72% for calcium, 5.81% for magnesium, 16.14% for manganese and 5.48% for potassium). The difference for zinc between the mean value for MON 88701 and the conventional control was a decrease of 5.81%. All of the nutrient mean values for MON 88701

observed in the combined-site analysis for minerals were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. With the exception of calcium and manganese, significant differences for mineral mean values between MON 88701 and the conventional control were not consistently observed among individual sites. Although calcium was significantly different at seven sites, with increases ranging from 9.33 to 24.41%, the variability observed for the test (mean range 0.10 to 0.21% dw) and control samples (mean range 0.081 to 0.19% dw) were very similar (Table E-20). Manganese was significantly different at five sites with increased differences ranging from 12.88 to 29.26%. Magnesium was significantly different at four sites with relative increases in MON 88701, ranging from 5.69 to 8.36%, and potassium was significantly increased at three sites, with relative increases ranging from 8.76 to 18.36%. Zinc was significantly different at two sites with small decreases in relative magnitude of differences ranging from 8.98 to 16.23%. Overall, observed differences in mineral values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were generally small, not consistently reproduced across individual sites (with the exception of calcium), and the mean MON 88701 combined-site values were within the 99% tolerance interval established by conventional, commercial reference varieties grown concurrently in the same trial or within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

The relative magnitude of the difference between the mean vitamin E value for MON 88701 and the conventional control in the combined-site analysis was a small increase of 5.84%. The mean vitamin E value for MON 88701 observed in the combined-site analysis was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. The significant difference for the vitamin E mean value between MON 88701 and the conventional control was not consistently observed among individual sites, with significant increases ranging from 6.54 to 11.36% observed at three sites. Overall, the observed difference in the vitamin E value between MON 88701 and the conventional control was not considered to be meaningful from a food and feed safety and nutritional perspective because it was small, not consistently reproduced across the individual sites, and the mean MON 88701 combined-site value was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and was within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

In summary, with the exception of calcium and manganese, statistical analyses found no consistent differences between the levels of nutrient components in cottonseed from MON 88701 and the conventional control. Differences were observed for calcium and manganese in combined analyses and most individual sites, but the magnitudes of differences for this nutrient was less than the variability for the control samples, and values were within the range of natural variability for cottonseed.

These findings support the conclusion of compositional equivalence of MON 88701 to conventional cotton.

E.5.2. Anti-Nutrient Levels in Cottonseed Not Treated with Dicamba or Glufosinate

Cottonseed was analyzed for five anti-nutrients, namely: dihydrosterculic acid, malvalic acid, sterculic acid, free gossypol, and total gossypol. Out of these five anti-nutrients, in the combined-site analysis of MON 88701 not treated with dicamba or glufosinate and the conventional control, malvalic and sterculic acids, as well as free gossypol, did not show any significant differences ($p<0.05$) in their mean values (Table E-21). In the combined-site analysis dihydrosterculic acid and total gossypol were significantly different (Table E-2).

The significant differences in anti-nutrients were further evaluated using considerations relevant to the safety and nutritional quality of MON 88701 when compared to the conventional control:

1) All anti-nutrient component differences observed in the combined-site statistical analysis, which reflected an increase in MON 88701 mean values with respect to the conventional control, were 12.64% or less. The relative magnitudes of the differences for dihydrosterculic acid and total gossypol were 12.64% and 6.26%, respectively.

2) Mean values for all significantly different anti-nutrient components from the combined-site analysis of MON 88701 were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial.

3) Assessment of the reproducibility of the combined-site differences at the eight individual sites showed significant differences for: dihydrosterculic and total gossypol at only one site. All individual site mean values of MON 88701 for both anti-nutrient components with significant differences were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial.

4) All combined-site mean values and individual site mean values of MON 88701 for all anti-nutrient components including those that were significantly different were within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

The two cottonseed anti-nutrient differences between MON 88701 and the conventional control observed in the combined-site data analysis were attributed to small differences in one cyclopropenoid fatty acid (dihydrosterculic; expressed as % total fatty acid) and total gossypol (expressed as % dw). The relative magnitude of the differences between the mean values for MON 88701 and the conventional control were increases of 12.64% for dihydrosterculic acid and 6.26% for total gossypol. These anti-nutrient mean values for MON 88701 observed in the combined-site analysis were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Significant differences for the two anti-nutrient mean values between MON 88701 and the conventional control were not consistently observed among individual sites. Both dihydrosterculic acid and total gossypol were significantly different at only one site with an increase of 33.11% for dihydrosterculic acid and an increase of 7.99% for total gossypol. Overall, observed differences in anti-nutrient values between MON 88701 and the conventional control were not considered to be meaningful from a food and feed safety or nutritional perspective because they were generally small, not

consistently reproduced across the individual sites, and the mean MON 88701 values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial and within the context of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2011).

In summary, statistical analyses found no consistent statistically significant differences between the levels of anti-nutrient components in cottonseed from MON 88701 and the conventional control and mean values for anti-nutrients were within the natural variability found for cottonseed. These findings supported the conclusion of compositional equivalence of MON 88701 to conventional cotton.

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Cottonseed Proximate (% dw)						
Ash	4.30	4.11	4.77	0.002	3.76 - 4.88	3.42, 4.65
Calories (Kcal/100g)	500.37	495.24	1.04	<0.001	487.62 - 511.92	457.61, 527.56
Carbohydrates	44.47	45.83	-2.96	<0.001	41.07 - 48.81	40.26, 56.45
Moisture (% fw)	7.00	7.48	-6.45	<0.001	5.81 - 9.07	4.79, 9.92
Total Fat	23.51	22.31	5.37	<0.001	20.99 - 25.54	15.01, 28.51
Cottonseed Fiber (% dw)						
Acid Detergent Fiber	25.53	26.58	-3.93	0.009	23.30 - 30.43	22.24, 31.96
Crude Fiber	17.78	18.54	-4.12	0.020	14.54 - 20.73	16.93, 22.68

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Cottonseed Fiber (% dw)						
Neutral Detergent Fiber	31.43	32.59	-3.56	0.005	28.05 - 37.27	27.03, 42.49
Total Dietary Fiber	39.75	41.12	-3.35	0.001	36.22 - 43.22	34.52, 52.58
Cottonseed Amino Acid (% dw)						
Arginine	3.03	3.15	-3.82	0.002	2.31 - 3.62	2.38, 3.47
Cottonseed Fatty Acid (% Total FA)						
14:0 Myristic	0.77	0.79	-2.39	0.018	0.66 - 0.95	0.16, 1.37
Cottonseed Mineral						
Calcium (% dw)	0.15	0.13	14.72	<0.001	0.10 - 0.21	0.058, 0.21
Magnesium (% dw)	0.40	0.38	5.81	<0.001	0.35 - 0.45	0.28, 0.47

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Cottonseed Mineral						
Manganese (mg/kg dw)	13.63	11.73	16.14	<0.001	10.59 - 17.47	9.07, 17.33
Potassium (% dw)	1.13	1.07	5.48	0.012	0.99 - 1.32	0.92, 1.21
Zinc (mg/kg dw)	37.81	40.14	-5.81	0.009	27.60 - 46.04	27.27, 44.95
Cottonseed Vitamin (mg/kg dw)						
Vitamin E	139.01	131.33	5.84	0.002	87.22 - 184.47	41.91, 205.89
Cottonseed Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15	0.14	12.64	<0.001	0.12 - 0.19	0.078, 0.25
Cottonseed Gossypol (% dw)						
Total Gossypol	1.03	0.97	6.26	<0.001	0.84 - 1.52	0.064, 1.76

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88701 minus Control) Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 7 Sites						
Calcium (% dw) Site ARTI	0.15	0.12	24.41	0.007	0.14 - 0.16	0.058, 0.21
Calcium (% dw) Site GACH	0.13	0.11	17.80	<0.001	0.13 - 0.13	0.058, 0.21
Calcium (% dw) Site KSLA	0.20	0.18	10.11	0.032	0.18 - 0.21	0.058, 0.21
Calcium (% dw) Site NCBD	0.15	0.14	9.33	0.002	0.15 - 0.15	0.058, 0.21
Calcium (% dw) Site NMLC	0.15	0.13	15.03	0.006	0.14 - 0.15	0.058, 0.21
Calcium (% dw) Site SCEK	0.11	0.091	21.62	0.013	0.10 - 0.12	0.058, 0.21
Calcium (% dw) Site TXPL	0.16	0.14	17.83	<0.001	0.16 - 0.17	0.058, 0.21

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Fatty Acid (% Total FA) - 5 Sites						
18:0 Stearic Site ARTI	2.65	2.51	5.54	0.035	2.59 - 2.71	1.98, 2.95
18:0 Stearic Site LACH	2.61	2.52	3.54	0.014	2.58 - 2.64	1.98, 2.95
18:0 Stearic Site NCBD	2.53	2.34	7.94	0.021	2.49 - 2.57	1.98, 2.95
18:0 Stearic Site NMLC	2.52	2.64	-4.70	<0.001	2.49 - 2.56	1.98, 2.95
18:0 Stearic Site TXPL	2.31	2.46	-6.14	0.001	2.26 - 2.34	1.98, 2.95
Cottonseed Mineral - 5 Sites						
Manganese (mg/kg dw) Site ARTI	14.86	11.50	29.26	0.034	13.28 - 17.47	9.07, 17.33
Manganese (mg/kg dw) Site GACH	14.26	11.51	23.89	<0.001	13.82 - 15.04	9.07, 17.33

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88701 minus Control) Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 5 Sites						
Manganese (mg/kg dw) Site KSLA	14.18	12.56	12.88	0.013	13.48 - 14.84	9.07, 17.33
Manganese (mg/kg dw) Site SCEK	12.44	9.72	28.04	0.008	10.59 - 13.87	9.07, 17.33
Manganese (mg/kg dw) Site TXPL	11.38	9.04	25.84	0.002	10.73 - 12.83	9.07, 17.33
Cottonseed Proximate (% dw) - 4 Sites						
Ash Site GACH	4.39	4.21	4.34	0.003	4.24 - 4.50	3.42, 4.65
Ash Site NCBD	4.33	4.14	4.56	0.043	4.20 - 4.48	3.42, 4.65
Ash Site SCEK	4.10	3.74	9.72	0.011	3.91 - 4.23	3.42, 4.65
Ash Site TXPL	3.84	3.46	11.00	0.001	3.76 - 3.98	3.42, 4.65

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Fatty Acid (% Total FA) - 4 Sites						
18:3 Linolenic Site ARTI	0.14	0.13	9.88	0.022	0.14 - 0.14	0.060, 0.24
18:3 Linolenic Site NCBD	0.36	0.29	25.90	0.008	0.34 - 0.38	0.060, 0.24
18:3 Linolenic Site NMLC	0.16	0.14	9.67	0.004	0.15 - 0.16	0.060, 0.24
18:3 Linolenic Site SCEK	0.16	0.15	6.70	0.005	0.15 - 0.16	0.060, 0.24
Cottonseed Mineral - 4 Sites						
Magnesium (% dw) Site GACH	0.41	0.38	8.36	<0.001	0.40 - 0.43	0.28, 0.47
Magnesium (% dw) Site KSLA	0.42	0.40	5.69	0.004	0.41 - 0.43	0.28, 0.47
Magnesium (% dw) Site SCEK	0.38	0.36	7.25	0.017	0.36 - 0.41	0.28, 0.47

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Mineral - 4 Sites						
Magnesium (% dw) Site TXPL	0.36	0.34	7.43	<0.001	0.35 - 0.37	0.28, 0.47
Cottonseed Fatty Acid (% Total FA) - 3 Sites						
14:0 Myristic Site KSLA	0.69	0.72	-4.64	0.013	0.66 - 0.70	0.16, 1.37
14:0 Myristic Site NCBD	0.71	0.75	-4.79	0.024	0.69 - 0.73	0.16, 1.37
14:0 Myristic Site NMLC	0.95	0.98	-3.08	0.008	0.94 - 0.95	0.16, 1.37
Cottonseed Mineral - 3 Sites						
Potassium (% dw) Site GACH	1.22	1.12	8.76	<0.001	1.18 - 1.24	0.92, 1.21
Potassium (% dw) Site SCEK	1.14	1.02	11.89	0.031	1.11 - 1.19	0.92, 1.21
Potassium (% dw) Site TXPL	1.03	0.87	18.36	0.002	0.99 - 1.09	0.92, 1.21

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Vitamin (mg/kg dw) - 3 Sites						
Vitamin E Site GACH	149.29	140.12	6.54	0.046	142.84 - 153.55	41.91, 205.89
Vitamin E Site LACH	167.00	149.96	11.36	0.002	158.95 - 173.30	41.91, 205.89
Vitamin E Site NCBD	174.64	156.99	11.24	0.025	165.41 - 184.47	41.91, 205.89
Cottonseed Proximate (% dw) - 2 Sites						
Moisture (% fw) Site GACH	6.39	7.23	-11.58	0.015	6.19 - 6.64	4.79, 9.92
Moisture (% fw) Site SCEK	6.48	7.08	-8.51	0.019	6.16 - 6.98	4.79, 9.92
Cottonseed Fiber (% dw) - 2 Sites						
Acid Detergent Fiber Site ARTI	25.07	27.53	-8.91	0.010	24.53 - 25.48	22.24, 31.96
Acid Detergent Fiber Site LACH	25.94	28.35	-8.50	0.008	25.56 - 26.33	22.24, 31.96

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Fiber (% dw) - 2 Sites						
Total Dietary Fiber Site LACH	41.09	43.35	-5.21	0.016	39.95 - 42.26	34.52, 52.58
Total Dietary Fiber Site NMLC	38.30	41.10	-6.83	0.003	37.12 - 39.32	34.52, 52.58
Cottonseed Amino Acid (% dw) - 2 Sites						
Arginine Site KSLA	3.00	3.28	-8.48	0.009	2.88 - 3.05	2.38, 3.47
Arginine Site NMLC	3.51	3.71	-5.36	0.008	3.34 - 3.62	2.38, 3.47
Lysine Site KSLA	1.19	1.25	-5.16	0.028	1.16 - 1.22	1.01, 1.30
Lysine Site LACH	1.25	1.18	6.25	0.035	1.23 - 1.27	1.01, 1.30
Cottonseed Fatty Acid (% Total FA) - 2 Sites						
18:2 Linoleic Site LACH	56.04	56.63	-1.03	0.027	55.71 - 56.35	47.49, 63.18

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Cottonseed Fatty Acid (% Total FA) - 2 Sites						
18:2 Linoleic Site NCBD	56.04	57.19	-2.01	0.021	55.76 - 56.31	47.49, 63.18
Cottonseed Mineral - 2 Sites						
Zinc (mg/kg dw) Site NCBD	41.50	49.54	-16.23	0.009	39.45 - 43.05	27.27, 44.95
Zinc (mg/kg dw) Site NMLC	44.99	49.43	-8.98	0.004	44.56 - 46.04	27.27, 44.95
Statistical Differences Observed in One Site						
Cottonseed Proximate (% dw)						
Calories (Kcal/100g) Site NCBD	498.21	491.80	1.30	0.048	496.22 - 499.62	457.61, 527.56
Carbohydrates Site TXPL	44.20	46.39	-4.71	0.014	43.36 - 44.54	40.26, 56.45
Total Fat Site NCBD	23.08	21.59	6.90	0.022	22.78 - 23.39	15.01, 28.51

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Site						
Cottonseed Fiber (% dw)						
Crude Fiber Site KSLA	16.71	17.67	-5.46	0.047	16.10 - 17.37	16.93, 22.68
Neutral Detergent Fiber Site LACH	32.12	34.05	-5.66	0.003	30.20 - 33.94	27.03, 42.49
Cottonseed Amino Acid (% dw)						
Aspartic Acid Site KSLA	2.35	2.50	-5.91	0.038	2.32 - 2.37	1.94, 2.57
Glutamic Acid Site GACH	4.69	4.96	-5.45	0.041	4.51 - 4.98	3.74, 5.28
Histidine Site KSLA	0.73	0.77	-6.41	0.006	0.69 - 0.76	0.59, 0.81
Isoleucine Site KSLA	0.90	0.95	-5.13	0.017	0.88 - 0.94	0.75, 0.96
Leucine Site KSLA	1.51	1.58	-4.68	0.017	1.47 - 1.55	1.25, 1.62

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Site						
Cottonseed Amino Acid (% dw)						
Phenylalanine Site KSLA	1.42	1.53	-6.88	0.014	1.37 - 1.45	1.12, 1.58
Tyrosine Site KSLA	0.79	0.84	-5.94	0.045	0.76 - 0.80	0.67, 0.84
Cottonseed Fatty Acid (% Total FA)						
18:1 Oleic Site NCBD	15.29	14.70	4.04	0.026	15.01 - 15.50	11.38, 20.64
22:0 Behenic Site ARTI	0.14	0.15	-6.75	0.029	0.14 - 0.15	0.070, 0.21
Cottonseed Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid Site GACH	0.16	0.12	33.11	0.012	0.14 - 0.17	0.078, 0.25
Sterculic Acid Site GACH	0.23	0.18	24.51	0.028	0.20 - 0.24	0.17, 0.27

Table E-19. Summary of Differences (p<0.05) for the Comparison of Cottonseed Component Levels for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88701 minus Control) Mean Difference (% of Control)	Significance (p-Value)	MON 88701 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Site						
Cottonseed Gossypol (% dw)						
Free Gossypol Site NMLC	0.86	0.69	23.91	0.008	0.76 - 0.95	0.099, 1.57
Total Gossypol Site TXPL	1.09	1.01	7.99	0.009	1.03 - 1.16	0.064, 1.76

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]Test refers to MON 88701 (Not Treated). These plants were not treated with dicamba or glufosinate.
[3]Mean = least-square mean.
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.30 (0.11) (3.76 - 4.88)	4.11 (0.11) (3.34 - 5.00)	0.20 (0.052) (-0.42 - 0.79)	0.084, 0.31	0.002	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	500.37 (1.65) (487.62 - 511.92)	495.24 (1.71) (487.70 - 512.65)	5.13 (1.29) (-5.97 - 15.41)	2.57, 7.69	<0.001	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.47 (0.56) (41.07 - 48.81)	45.83 (0.57) (42.14 - 50.30)	-1.36 (0.32) (-5.09 - 1.56)	-1.99, -0.73	<0.001	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	7.00 (0.26) (5.81 - 9.07)	7.48 (0.27) (6.15 - 9.19)	-0.48 (0.11) (-1.45 - 0.15)	-0.71, -0.26	<0.001	4.79, 9.92 (6.05 - 10.50)
Protein	27.71 (0.77) (22.49 - 31.29)	27.79 (0.77) (23.53 - 31.27)	-0.075 (0.31) (-2.53 - 4.39)	-0.73, 0.58	0.810	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.51 (0.31) (20.99 - 25.54)	22.31 (0.33) (20.71 - 25.20)	1.20 (0.26) (-1.21 - 3.21)	0.69, 1.71	<0.001	15.01, 28.51 (16.58 - 25.25)

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.53 (0.34) (23.30 - 30.43)	26.58 (0.35) (22.08 - 29.58)	-1.05 (0.35) (-4.02 - 5.70)	-1.79, -0.30	0.009	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.78 (0.37) (14.54 - 20.73)	18.54 (0.38) (16.06 - 21.70)	-0.76 (0.32) (-5.57 - 3.82)	-1.41, -0.12	0.020	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	31.43 (0.51) (28.05 - 37.27)	32.59 (0.53) (28.87 - 35.89)	-1.16 (0.41) (-4.66 - 6.42)	-1.97, -0.35	0.005	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.75 (0.39) (36.22 - 43.22)	41.12 (0.41) (39.05 - 44.37)	-1.38 (0.36) (-4.41 - 2.04)	-2.15, -0.61	0.001	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.05 (0.020) (0.88 - 1.15)	1.05 (0.020) (0.88 - 1.17)	-0.0034 (0.0091) (-0.076 - 0.11)	-0.023, 0.016	0.714	0.86, 1.11 (0.83 - 1.22)
Arginine	3.03 (0.10) (2.31 - 3.62)	3.15 (0.10) (2.41 - 3.77)	-0.12 (0.033) (-0.37 - 0.26)	-0.19, -0.050	0.002	2.38, 3.47 (2.30 - 3.55)

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.39 (0.062) (1.95 - 2.69)	2.41 (0.062) (1.92 - 2.74)	-0.022 (0.027) (-0.28 - 0.20)	-0.079, 0.035	0.422	1.94, 2.57 (1.79 - 2.72)
Cystine	0.40 (0.0091) (0.33 - 0.49)	0.40 (0.0094) (0.31 - 0.46)	-0.0018 (0.0070) (-0.074 - 0.086)	-0.016, 0.012	0.793	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.71 (0.13) (3.79 - 5.57)	4.84 (0.14) (3.66 - 5.70)	-0.13 (0.072) (-1.01 - 0.57)	-0.29, 0.025	0.093	3.74, 5.28 (3.39 - 5.45)
Glycine	1.09 (0.020) (0.92 - 1.19)	1.09 (0.020) (0.91 - 1.20)	-0.0061 (0.011) (-0.11 - 0.088)	-0.029, 0.017	0.577	0.90, 1.14 (0.85 - 1.23)
Histidine	0.74 (0.019) (0.58 - 0.84)	0.75 (0.019) (0.61 - 0.84)	-0.0076 (0.0073) (-0.069 - 0.064)	-0.023, 0.0079	0.312	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.91 (0.018) (0.76 - 1.01)	0.92 (0.018) (0.77 - 1.03)	-0.0087 (0.0079) (-0.074 - 0.074)	-0.026, 0.0082	0.291	0.75, 0.96 (0.72 - 1.03)

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.53 (0.032) (1.28 - 1.68)	1.54 (0.032) (1.28 - 1.69)	-0.0080 (0.013) (-0.095 - 0.14)	-0.036, 0.020	0.544	1.25, 1.62 (1.20 - 1.72)
Lysine	1.23 (0.025) (1.03 - 1.37)	1.23 (0.025) (1.06 - 1.39)	-0.0019 (0.015) (-0.084 - 0.14)	-0.034, 0.031	0.904	1.01, 1.30 (0.99 - 1.44)
Methionine	0.39 (0.0079) (0.33 - 0.44)	0.38 (0.0084) (0.32 - 0.46)	0.011 (0.0081) (-0.081 - 0.088)	-0.0049, 0.027	0.167	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.43 (0.039) (1.13 - 1.63)	1.46 (0.039) (1.15 - 1.66)	-0.026 (0.014) (-0.14 - 0.11)	-0.056, 0.0044	0.088	1.12, 1.58 (1.10 - 1.63)
Proline	1.02 (0.029) (0.78 - 1.16)	1.03 (0.029) (0.81 - 1.25)	-0.014 (0.012) (-0.13 - 0.10)	-0.039, 0.011	0.246	0.83, 1.08 (0.79 - 1.17)
Serine	1.08 (0.025) (0.93 - 1.28)	1.09 (0.026) (0.86 - 1.24)	-0.0031 (0.015) (-0.14 - 0.14)	-0.035, 0.028	0.834	0.83, 1.21 (0.81 - 1.24)

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.87 (0.016) (0.73 - 0.95)	0.86 (0.016) (0.73 - 0.95)	0.0033 (0.0083) (-0.062 - 0.086)	-0.014, 0.021	0.694	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.0092) (0.34 - 0.50)	0.42 (0.0095) (0.37 - 0.52)	-0.0092 (0.0066) (-0.099 - 0.11)	-0.022, 0.0041	0.171	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.81 (0.017) (0.68 - 0.88)	0.81 (0.018) (0.67 - 0.91)	-0.0030 (0.0083) (-0.076 - 0.074)	-0.021, 0.015	0.718	0.67, 0.84 (0.63 - 0.91)
Valine	1.21 (0.027) (0.98 - 1.38)	1.23 (0.027) (1.00 - 1.40)	-0.013 (0.011) (-0.10 - 0.15)	-0.037, 0.011	0.257	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.77 (0.030) (0.66 - 0.95)	0.79 (0.031) (0.71 - 0.98)	-0.019 (0.0071) (-0.087 - 0.041)	-0.034, -0.0036	0.018	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	23.93 (0.30) (22.30 - 25.45)	23.80 (0.30) (22.69 - 25.05)	0.13 (0.076) (-0.48 - 0.63)	-0.035, 0.29	0.116	16.54, 30.55 (19.11 - 26.73)

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.50 (0.0094) (0.45 - 0.55)	0.50 (0.0094) (0.45 - 0.54)	-0.00052 (0.0038) (-0.024 - 0.024)	-0.0087, 0.0077	0.894	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.52 (0.058) (2.16 - 2.93)	2.47 (0.058) (2.15 - 2.76)	0.045 (0.036) (-0.24 - 0.26)	-0.032, 0.12	0.228	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	15.05 (0.26) (14.05 - 16.29)	14.96 (0.26) (14.06 - 16.44)	0.094 (0.070) (-0.54 - 1.00)	-0.055, 0.24	0.196	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.84 (0.39) (54.22 - 58.48)	56.15 (0.40) (54.04 - 57.93)	-0.31 (0.16) (-1.65 - 0.73)	-0.64, 0.023	0.065	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.18 (0.022) (0.11 - 0.38)	0.17 (0.022) (0.12 - 0.30)	0.013 (0.0068) (-0.032 - 0.11)	-0.0017, 0.027	0.078	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.29 (0.0086) (0.23 - 0.32)	0.28 (0.0087) (0.23 - 0.32)	0.0044 (0.0047) (-0.049 - 0.042)	-0.0057, 0.015	0.365	0.17, 0.38 (0.20 - 0.36)

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.15 (0.0051) (0.12 - 0.17)	0.15 (0.0051) (0.13 - 0.21)	-0.0010 (0.0029) (-0.057 - 0.027)	-0.0074, 0.0053	0.730	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0093) (0.10 - 0.21)	0.13 (0.0093) (0.081 - 0.19)	0.019 (0.0022) (0.0037 - 0.039)	0.014, 0.024	<0.001	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	8.94 (0.70) (5.02 - 12.15)	8.93 (0.70) (5.40 - 11.92)	0.015 (0.16) (-2.19 - 1.72)	-0.30, 0.33	0.925	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	72.43 (4.40) (41.73 - 109.70)	71.33 (4.48) (45.03 - 95.10)	1.10 (2.74) (-20.63 - 27.89)	-4.74, 6.94	0.693	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.40 (0.0083) (0.35 - 0.45)	0.38 (0.0084) (0.33 - 0.44)	0.022 (0.0032) (-0.015 - 0.055)	0.016, 0.028	<0.001	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	13.63 (0.47) (10.59 - 17.47)	11.73 (0.48) (8.61 - 14.11)	1.89 (0.28) (-0.84 - 5.26)	1.29, 2.50	<0.001	9.07, 17.33 (9.07 - 17.14)

Table E-20. Statistical Summary of Combined-Site Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.71 (0.031) (0.58 - 0.87)	0.72 (0.031) (0.54 - 0.87)	-0.0035 (0.0067) (-0.078 - 0.072)	-0.017, 0.0099	0.605	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.13 (0.028) (0.99 - 1.32)	1.07 (0.028) (0.79 - 1.27)	0.059 (0.020) (-0.13 - 0.31)	0.015, 0.10	0.012	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.026 (0.0095) (0.0053 - 0.082)	0.029 (0.0096) (0.0053 - 0.10)	-0.0035 (0.0046) (-0.085 - 0.026)	-0.013, 0.0064	0.466	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	37.81 (2.01) (27.60 - 46.04)	40.14 (2.02) (28.22 - 52.95)	-2.33 (0.77) (-13.50 - 1.99)	-3.99, -0.67	0.009	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	139.01 (9.87) (87.22 - 184.47)	131.33 (9.88) (91.78 - 162.98)	7.68 (2.07) (-7.82 - 32.93)	3.26, 12.09	0.002	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]Test refers to MON 88701 (Not Treated). These plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-21. Statistical Summary of Combined-Site Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0034) (0.12 - 0.19)	0.14 (0.0037) (0.11 - 0.17)	0.017 (0.0044) (-0.011 - 0.058)	0.0086, 0.026	<0.001	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.39 (0.015) (0.24 - 0.50)	0.37 (0.016) (0.26 - 0.49)	0.019 (0.015) (-0.11 - 0.19)	-0.011, 0.048	0.210	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.22 (0.0067) (0.12 - 0.27)	0.21 (0.0072) (0.17 - 0.27)	0.0099 (0.0081) (-0.075 - 0.093)	-0.0064, 0.026	0.229	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.93 (0.037) (0.76 - 1.10)	0.89 (0.037) (0.68 - 1.20)	0.041 (0.020) (-0.14 - 0.27)	-0.0031, 0.084	0.065	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.03 (0.037) (0.84 - 1.52)	0.97 (0.037) (0.74 - 1.10)	0.061 (0.017) (-0.028 - 0.44)	0.026, 0.096	<0.001	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2]Test refers to MON 88701 (Not Treated). These plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-22 Literature and ILSI Ranges for Components in Cottonseed

Cottonseed Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Cottonseed Nutrients		
Proximates (% dw)		
Ash	3.87 – 5.29[a]; 3.7 – 4.2[d]	3.761 – 5.342
Carbohydrates by calculation	45.28 – 53.62[a]	39.0 – 53.6
Calories by calculation (Kcal/100g)	471.34 – 506.95[a]	Not available
Moisture (% fw)	2.25 – 7.49[a]	2.3 – 9.9
Protein	24.54 – 30.83[a]; 21.2 – 25.9[b]	21.48 – 32.97
Total Fat	17.37 – 25.16[a]; 14.4 – 16.9[d]	17.201 – 27.292
Fiber (% dw)		
Acid Detergent Fiber	21.10 – 34.8[a]; 37.6 – 40.5[d]	19.74 – 38.95
Neutral Detergent Fiber	32.92 – 45.83[a]; 50.0 – 53.6[d]	25.56 – 51.87
Crude Fiber	13.85 – 17.94[a]	13.86 – 23.10
Total Dietary Fiber	not available	33.69 – 47.55
Amino Acids	**(% total AA)**	**(% dw)**
Alanine	4.16 – 4.41[a]; 3.6 – 4.2[b]	0.80 – 1.22
Arginine	11.28 – 12.51[a]; 10.9 – 12.3[b]	2.06 – 3.72
Aspartic acid	9.73 – 9.99[a]; 8.8 – 9.5[b]	1.82 – 2.94
Cystine/Cysteine	1.60 – 1.92[a]; 2.3 – 3.4[b]	0.35 – 0.56
Glutamic acid	20.76 – 21.61[a]; 20.5 – 22.4[b]	3.91 – 6.72
Glycine	4.44 – 4.58[a]; 3.8 – 4.5[b]	0.83 – 1.32
Histidine	3.00 – 3.12[a]; 2.6 – 2.8[b]	0.57 – 0.91
Isoleucine	3.10 – 3.67[a]; 3.0 – 3.4[b]	0.62 – 1.05
Leucine	6.27 – 6.65[a]; 5.5 – 6.1[b]	1.14 – 1.86
Lysine	4.85 – 5.37[a]; 4.2 – 4.6[b]	0.94 – 1.46
Methionine	1.46 – 1.88[a]; 1.3 – 1.8[b]	0.30 – 0.47
Phenylalanine	5.56 – 5.77[a]; 5.0 – 5.6[b]	1.02 – 1.72
Proline	4.06 – 4.28[a]; 3.1 – 4.0[b]	0.75 – 1.23
Serine	4.45 – 4.86[a]; 3.9 – 4.4[b]	0.91 – 1.35
Threonine	3.26 – 3.59[a]; 2.8 – 3.2[b]	0.55 – 0.92
Tryptophan	0.97 – 1.21[a]; 1.0 – 1.4[b]	0.194 – 0.319
Tyrosine	2.65 – 2.92[a]; 2.8 – 3.3[b]	0.53 – 0.84
Valine	4.76 – 5.14[a]; 4.3 – 4.7[b]	0.87 – 1.49
Fatty Acids (% total FA)		
8:0 Caprylic	not available	not available
10:0 Capric	not available	not available
12:0 Lauric	not available	not available
14:0 Myristic	0.55 – 2.40[a]; 0.6 – 1.5[b]	0.455 – 2.400
14:1 Myristoleic	not available	not available
15:0 Pentadecanoic	0.050 – 0.17[a]	0.103 – 0.481
15:1 Pentadecenoic	not available	not available
16:0 Palmitic	21.23 – 27.9[a]; 17.6 – 24.8[b]	15.11 – 27.90
16:1 Palmitoleic	0.55 – 1.16[a]	0.464 – 1.190
17:0 Heptadecanoic	not available	0.092 – 0.119

Table E-22. Literature and ILSI Ranges for Components in Cottonseed (continued)

Cottonseed Tissue Components[1]	Literature Range[2]	ILSI Range[3]
17:1 Heptadecenoic	not available	not available
18:0 Stearic	1.99 – 3.11 [a]; 2.0 – 2.5 [b]	0.20 – 3.11
18:1 Oleic	13.90 – 20.10 [a]; 15.0 – 20.7 [b]	12.8 – 25.3
18:2 Linoleic	46.00 – 56.88 [a]	46.0 – 59.4
18:3 Gamma Linolenic	0.050 – 0.25 [a]	0.097 – 0.232
18:3 Linolenic	0.050 – 0.25 [a]	0.11 – 0.35
20:0 Arachidic	0.25 – 0.33 [a]	0.186 – 0.414
20:1 Eicosenoic	not available	0.095 – 0.098
20:2 Eicosadienoic	not available	not available
20:3 Eicosatrienoic	not available	not available
20:4 Arachidonic	not available	not available
22:0 Behenic	0.13 – 0.17 [a]	0.104 – 0.295
Vitamins	**(mg/kg fw)**	**(mg/kg dw)**
Vitamin E	99 – 224[c]	70.825 – 197.243
Minerals (% dw)		
Calcium	0.10 – 0.33[a]	0.10323 – 0.32581
Copper (mg/kg dw)	3.54 – 11.14[a]	3.13 – 24.57
Iron (mg/kg dw)	40.58 – 56.54 [a]	36.71 – 318.38
Magnesium	0.37 – 0.46 [a]	0.34709 – 0.49312
Manganese (mg/kg dw)	11.06 – 18.31 [a]	10.69 – 21.96
Phosphorus	0.60 – 0.84 [a]	0.48254 – 0.99157
Potassium	0.98 – 1.24 [a]	0.98345 – 1.44835
Sodium	0.0054 – 0.74 [a]	0.01118 – 0.73548
Zinc (mg/kg dw)	30.21 – 47.75 [a]	27.0 – 59.5
Cottonseed Anti-Nutrients		
Gossypol, Total (% dw)	0.57 – 1.42[a]; 0.55 – 0.77[d]	0.547 – 1.522
Gossypol, Free (% dw)	0.53 – 1.20[a]	0.454 – 1.399
Cyclopropenoid Fatty Acids (% total FA)		
Dihydrosterculic	0.13 – 0.24 [a]	0.075 – 0.310
Malvalic	0.33 – 0.58 [a]	0.229 – 0.759
Sterculic	0.21 – 0.56 [a]	0.190 – 0.556

[1] fw=fresh weight; dw=dry weight
[2] Literature range references: [a](Hamilton, et al., 2004); [b](Lawhon, et al., 1977); [c](Smith and Creelman, 2001); [d](Bertrand, et al., 2005).
[3] (ILSI, 2011).

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.71 (0.087) (4.58 - 4.88)	4.74 (0.12) (4.49 - 5.00)	-0.030 (0.15) (-0.42 - 0.39)	-0.45, 0.39	0.850	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	499.07 (3.00) (493.81 - 501.81)	488.30 (3.88) (487.70 - 494.60)	10.76 (3.90) (7.21 - 13.11)	-0.069, 21.59	0.050	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.29 (0.58) (43.36 - 45.15)	46.35 (0.81) (45.03 - 47.37)	-2.06 (0.96) (-2.98 - -1.67)	-4.72, 0.60	0.097	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	7.08 (0.27) (6.14 - 7.86)	7.63 (0.35) (7.32 - 7.40)	-0.55 (0.35) (-1.26 - -0.37)	-1.51, 0.41	0.184	4.79, 9.92 (6.05 - 10.50)
Protein	27.39 (0.24) (26.65 - 28.02)	27.04 (0.33) (26.97 - 27.11)	0.35 (0.41) (-0.32 - 0.91)	-0.79, 1.48	0.442	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.62 (0.59) (22.57 - 24.08)	21.50 (0.78) (21.15 - 22.89)	2.12 (0.81) (1.18 - 2.93)	-0.12, 4.36	0.058	15.01, 28.51 (16.58 - 25.25)

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.07 (0.32) (24.53 - 25.48)	27.53 (0.45) (26.57 - 28.49)	-2.45 (0.55) (-3.34 - -1.42)	-3.97, -0.94	0.010	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.00 (0.90) (14.54 - 18.91)	19.47 (1.20) (19.33 - 19.85)	-2.47 (1.26) (-2.18 - -0.94)	-5.97, 1.03	0.121	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	31.02 (0.79) (29.94 - 32.82)	32.89 (1.06) (30.67 - 34.42)	-1.86 (1.13) (-3.04 - -0.73)	-5.01, 1.28	0.175	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.70 (1.06) (36.22 - 42.86)	41.67 (1.50) (40.50 - 42.84)	-1.97 (1.83) (-4.27 - -4.04)	-7.06, 3.12	0.343	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.03 (0.015) (1.00 - 1.05)	1.02 (0.018) (0.99 - 1.05)	0.0099 (0.017) (0.0033 - 0.028)	-0.037, 0.057	0.591	0.86, 1.11 (0.83 - 1.22)
Arginine	2.93 (0.052) (2.82 - 3.03)	3.02 (0.073) (2.89 - 3.13)	-0.086 (0.084) (-0.21 - 0.068)	-0.32, 0.15	0.362	2.38, 3.47 (2.30 - 3.55)

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.30 (0.045) (2.23 - 2.36)	2.32 (0.060) (2.19 - 2.42)	-0.024 (0.065) (-0.085 - 0.077)	-0.20, 0.16	0.729	1.94, 2.57 (1.79 - 2.72)
Cystine	0.38 (0.010) (0.36 - 0.40)	0.37 (0.014) (0.35 - 0.39)	0.016 (0.017) (0.0017 - 0.039)	-0.032, 0.064	0.406	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.52 (0.099) (4.29 - 4.73)	4.51 (0.14) (4.34 - 4.67)	0.0076 (0.17) (0.057 - 0.11)	-0.46, 0.47	0.965	3.74, 5.28 (3.39 - 5.45)
Glycine	1.07 (0.020) (1.04 - 1.10)	1.08 (0.026) (1.03 - 1.11)	-0.0049 (0.028) (-0.015 - 0.029)	-0.084, 0.074	0.872	0.90, 1.14 (0.85 - 1.23)
Histidine	0.71 (0.016) (0.66 - 0.74)	0.74 (0.023) (0.71 - 0.77)	-0.021 (0.026) (-0.042 - 0.034)	-0.094, 0.053	0.474	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.88 (0.018) (0.82 - 0.93)	0.91 (0.024) (0.88 - 0.93)	-0.029 (0.025) (-0.054 - 0.0021)	-0.098, 0.040	0.304	0.75, 0.96 (0.72 - 1.03)

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.48 (0.027) (1.41 - 1.55)	1.50 (0.037) (1.44 - 1.55)	-0.017 (0.042) (-0.071 - 0.045)	-0.13, 0.098	0.700	1.25, 1.62 (1.20 - 1.72)
Lysine	1.20 (0.044) (1.04 - 1.31)	1.23 (0.058) (1.19 - 1.26)	-0.032 (0.062) (-0.016 - -0.0053)	-0.21, 0.14	0.633	1.01, 1.30 (0.99 - 1.44)
Methionine	0.38 (0.015) (0.36 - 0.40)	0.35 (0.021) (0.35 - 0.36)	0.027 (0.025) (0.036 - 0.050)	-0.043, 0.097	0.347	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.39 (0.027) (1.34 - 1.43)	1.41 (0.038) (1.34 - 1.48)	-0.022 (0.045) (-0.10 - 0.067)	-0.15, 0.10	0.649	1.12, 1.58 (1.10 - 1.63)
Proline	0.97 (0.021) (0.89 - 1.03)	1.00 (0.028) (0.98 - 1.02)	-0.023 (0.030) (-0.044 - 0.0082)	-0.11, 0.061	0.490	0.83, 1.08 (0.79 - 1.17)
Serine	1.05 (0.022) (1.02 - 1.07)	1.03 (0.031) (0.99 - 1.06)	0.022 (0.038) (0.011 - 0.078)	-0.083, 0.13	0.590	0.83, 1.21 (0.81 - 1.24)

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.85 (0.012) (0.82 - 0.86)	0.85 (0.017) (0.82 - 0.88)	-0.00041 (0.021) (-0.028 - 0.040)	-0.058, 0.057	0.985	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.0062) (0.41 - 0.42)	0.42 (0.0087) (0.40 - 0.44)	-0.0085 (0.011) (-0.033 - 0.012)	-0.038, 0.021	0.468	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.80 (0.015) (0.76 - 0.83)	0.79 (0.021) (0.76 - 0.82)	0.0015 (0.026) (-0.034 - 0.043)	-0.071, 0.074	0.957	0.67, 0.84 (0.63 - 0.91)
Valine	1.17 (0.021) (1.12 - 1.23)	1.21 (0.027) (1.17 - 1.24)	-0.034 (0.026) (-0.059 - -0.0050)	-0.11, 0.039	0.263	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.78 (0.0085) (0.76 - 0.80)	0.78 (0.012) (0.77 - 0.78)	-0.0013 (0.013) (-0.0043 - 0.0026)	-0.037, 0.035	0.927	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	25.06 (0.10) (24.85 - 25.45)	24.98 (0.14) (24.92 - 25.05)	0.075 (0.18) (0.012 - 0.41)	-0.41, 0.56	0.692	16.54, 30.55 (19.11 - 26.73)

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.54 (0.0052) (0.52 - 0.55)	0.52 (0.0073) (0.52 - 0.52)	0.018 (0.0089) (0.016 - 0.024)	-0.0064, 0.043	0.107	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.65 (0.026) (2.59 - 2.71)	2.51 (0.036) (2.45 - 2.57)	0.14 (0.045) (0.10 - 0.26)	0.015, 0.26	0.035	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.82 (0.11) (14.65 - 14.99)	14.68 (0.15) (14.58 - 14.70)	0.13 (0.17) (-0.050 - 0.37)	-0.33, 0.60	0.475	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	54.83 (0.22) (54.28 - 55.22)	55.31 (0.31) (55.26 - 55.38)	-0.49 (0.37) (-1.09 - -0.074)	-1.52, 0.55	0.263	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.14 (0.0021) (0.14 - 0.14)	0.13 (0.0030) (0.12 - 0.14)	0.013 (0.0035) (0.0051 - 0.019)	0.0030, 0.023	0.022	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.31 (0.0079) (0.28 - 0.32)	0.29 (0.011) (0.27 - 0.31)	0.015 (0.011) (0.0037 - 0.012)	-0.017, 0.046	0.266	0.17, 0.38 (0.20 - 0.36)

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.14 (0.0026) (0.14 - 0.15)	0.15 (0.0033) (0.15 - 0.16)	-0.010 (0.0031) (-0.018 - -0.0043)	-0.019, -0.0016	0.029	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0035) (0.14 - 0.16)	0.12 (0.0050) (0.12 - 0.13)	0.030 (0.0061) (0.024 - 0.039)	0.013, 0.047	0.007	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	9.81 (0.34) (9.20 - 10.96)	9.64 (0.41) (8.79 - 9.79)	0.17 (0.38) (-0.45 - 0.41)	-0.88, 1.21	0.680	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	86.87 (5.63) (72.55 - 103.10)	80.76 (7.78) (72.89 - 87.72)	6.11 (8.79) (0.83 - 10.40)	-18.31, 30.53	0.525	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.43 (0.0076) (0.41 - 0.45)	0.40 (0.010) (0.38 - 0.41)	0.028 (0.011) (0.013 - 0.037)	-0.0019, 0.058	0.059	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	14.86 (0.63) (13.28 - 17.47)	11.50 (0.89) (11.34 - 11.55)	3.36 (1.06) (2.51 - 3.29)	0.41, 6.32	0.034	9.07, 17.33 (9.07 - 17.14)

Table E-23. Statistical Summary of Site ARTI Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.84 (0.012) (0.80 - 0.87)	0.84 (0.016) (0.82 - 0.87)	-0.0071 (0.018) (-0.027 - 0.012)	-0.058, 0.044	0.717	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.22 (0.029) (1.16 - 1.32)	1.11 (0.040) (1.06 - 1.15)	0.12 (0.047) (0.092 - 0.11)	-0.013, 0.25	0.067	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.017 (0.0038) (0.0054 - 0.030)	(0.013 - 0.013)			ND[6]	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	39.24 (1.65) (35.79 - 45.91)	40.81 (2.03) (38.63 - 40.71)	-1.56 (1.87) (-2.84 - -2.25)	-6.76, 3.63	0.449	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	141.82 (2.44) (132.11 - 146.82)	136.55 (3.46) (133.79 - 139.31)	5.27 (4.23) (-7.20 - 12.36)	-6.49, 17.02	0.281	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.
[6]Not determined due to insufficient number of observations for the control.

Table E-24. Statistical Summary of Site ARTI Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0073) (0.14 - 0.18)	0.15 (0.010) (0.14 - 0.15)	0.0079 (0.013) (-0.011 - 0.0068)	-0.027, 0.043	0.567	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.37 (0.016) (0.33 - 0.43)	0.36 (0.023) (0.33 - 0.37)	0.011 (0.025) (-0.0096 - 0.012)	-0.058, 0.081	0.674	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.014) (0.17 - 0.27)	0.20 (0.020) (0.19 - 0.20)	0.030 (0.023) (-0.013 - 0.022)	-0.035, 0.094	0.272	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.84 (0.035) (0.78 - 0.92)	0.82 (0.049) (0.80 - 0.84)	0.020 (0.060) (-0.021 - -0.0027)	-0.15, 0.19	0.749	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.93 (0.034) (0.87 - 1.04)	0.93 (0.044) (0.94 - 0.98)	0.0038 (0.044) (-0.0059 - 0.063)	-0.12, 0.13	0.934	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.39 (0.044) (4.24 - 4.50)	4.21 (0.047) (4.12 - 4.23)	0.18 (0.034) (0.12 - 0.25)	0.095, 0.27	0.003	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	501.28 (2.28) (498.88 - 503.43)	496.55 (2.63) (494.57 - 498.27)	4.73 (3.48) (2.32 - 6.61)	-4.23, 13.69	0.232	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.81 (0.59) (44.28 - 45.20)	45.84 (0.68) (44.64 - 47.09)	-1.03 (0.91) (-1.89 - -0.36)	-3.36, 1.30	0.306	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.39 (0.15) (6.19 - 6.64)	7.23 (0.18) (6.99 - 7.48)	-0.84 (0.23) (-0.97 - -0.57)	-1.43, -0.24	0.015	4.79, 9.92 (6.05 - 10.50)
Protein	27.03 (0.33) (26.24 - 28.02)	27.30 (0.37) (26.45 - 28.21)	-0.27 (0.37) (-1.02 - 0.63)	-1.21, 0.67	0.493	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.77 (0.46) (23.35 - 24.21)	22.67 (0.53) (22.26 - 23.02)	1.10 (0.70) (0.40 - 1.47)	-0.71, 2.91	0.180	15.01, 28.51 (16.58 - 25.25)

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	26.12 (0.52) (24.62 - 27.38)	27.52 (0.60) (26.81 - 28.13)	-1.40 (0.79) (-3.01 - 0.58)	-3.43, 0.63	0.136	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	18.88 (0.68) (17.57 - 19.90)	19.92 (0.78) (18.70 - 21.18)	-1.04 (1.00) (-2.85 - 1.20)	-3.60, 1.53	0.346	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	32.42 (0.90) (30.60 - 34.01)	33.92 (1.04) (32.79 - 35.89)	-1.49 (1.38) (-1.93 - 0.94)	-5.03, 2.05	0.327	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	39.71 (0.86) (37.96 - 42.04)	41.11 (1.00) (39.89 - 42.04)	-1.39 (1.32) (-3.36 - 0.94)	-4.79, 2.00	0.339	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.06 (0.019) (1.02 - 1.10)	1.10 (0.022) (1.06 - 1.17)	-0.034 (0.025) (-0.076 - 0.029)	-0.098, 0.030	0.234	0.86, 1.11 (0.83 - 1.22)
Arginine	3.06 (0.061) (2.96 - 3.18)	3.21 (0.067) (3.07 - 3.46)	-0.16 (0.064) (-0.28 - -0.062)	-0.32, 0.0098	0.060	2.38, 3.47 (2.30 - 3.55)

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.39 (0.043) (2.30 - 2.49)	2.45 (0.047) (2.36 - 2.60)	-0.066 (0.044) (-0.11 - 0.00069)	-0.18, 0.047	0.192	1.94, 2.57 (1.79 - 2.72)
Cystine	0.41 (0.012) (0.38 - 0.43)	0.40 (0.013) (0.38 - 0.43)	0.0020 (0.018) (-0.032 - 0.050)	-0.043, 0.047	0.913	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.69 (0.098) (4.51 - 4.98)	4.96 (0.11) (4.77 - 5.21)	-0.27 (0.099) (-0.26 - -0.23)	-0.52, -0.016	0.041	3.74, 5.28 (3.39 - 5.45)
Glycine	1.11 (0.020) (1.06 - 1.14)	1.13 (0.023) (1.09 - 1.20)	-0.016 (0.026) (-0.055 - 0.033)	-0.083, 0.050	0.559	0.90, 1.14 (0.85 - 1.23)
Histidine	0.76 (0.013) (0.73 - 0.78)	0.76 (0.014) (0.75 - 0.78)	-0.00082 (0.012) (-0.018 - 0.0081)	-0.033, 0.031	0.949	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.95 (0.010) (0.91 - 0.97)	0.94 (0.012) (0.92 - 0.97)	0.00070 (0.014) (-0.012 - 0.011)	-0.035, 0.036	0.961	0.75, 0.96 (0.72 - 1.03)

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.56 (0.022) (1.51 - 1.60)	1.58 (0.024) (1.52 - 1.65)	-0.019 (0.021) (-0.049 - 0.0079)	-0.074, 0.037	0.425	1.25, 1.62 (1.20 - 1.72)
Lysine	1.26 (0.018) (1.19 - 1.29)	1.23 (0.020) (1.22 - 1.25)	0.029 (0.024) (-0.040 - 0.062)	-0.033, 0.091	0.286	1.01, 1.30 (0.99 - 1.44)
Methionine	0.40 (0.017) (0.38 - 0.42)	0.42 (0.019) (0.37 - 0.46)	-0.019 (0.024) (-0.081 - 0.035)	-0.080, 0.042	0.464	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.45 (0.030) (1.41 - 1.49)	1.49 (0.033) (1.41 - 1.61)	-0.040 (0.032) (-0.12 - 0.0089)	-0.12, 0.041	0.259	1.12, 1.58 (1.10 - 1.63)
Proline	1.03 (0.020) (0.97 - 1.10)	1.05 (0.022) (1.03 - 1.09)	-0.021 (0.022) (-0.065 - 0.010)	-0.078, 0.036	0.393	0.83, 1.08 (0.79 - 1.17)
Serine	1.08 (0.031) (1.02 - 1.14)	1.12 (0.035) (1.08 - 1.20)	-0.040 (0.039) (-0.055 - 0.023)	-0.14, 0.061	0.357	0.83, 1.21 (0.81 - 1.24)

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.88 (0.015) (0.85 - 0.91)	0.90 (0.017) (0.87 - 0.95)	-0.017 (0.018) (-0.040 - 0.016)	-0.063, 0.029	0.395	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.011) (0.39 - 0.42)	0.42 (0.013) (0.39 - 0.44)	-0.016 (0.017) (-0.042 - 0.030)	-0.061, 0.028	0.389	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.84 (0.011) (0.82 - 0.85)	0.84 (0.013) (0.81 - 0.89)	-0.0011 (0.013) (-0.036 - 0.027)	-0.034, 0.032	0.936	0.67, 0.84 (0.63 - 0.91)
Valine	1.24 (0.018) (1.19 - 1.28)	1.26 (0.019) (1.23 - 1.32)	-0.020 (0.015) (-0.040 - 0.011)	-0.059, 0.018	0.235	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.77 (0.0068) (0.76 - 0.79)	0.77 (0.0079) (0.76 - 0.78)	-0.0036 (0.010) (-0.026 - 0.022)	-0.030, 0.023	0.745	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.27 (0.14) (24.04 - 24.70)	24.12 (0.16) (23.78 - 24.45)	0.15 (0.20) (-0.20 - 0.26)	-0.37, 0.67	0.494	16.54, 30.55 (19.11 - 26.73)

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.51 (0.0058) (0.50 - 0.52)	0.51 (0.0067) (0.50 - 0.52)	-0.0013 (0.0089) (-0.0059 - 0.013)	-0.024, 0.022	0.886	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.50 (0.032) (2.45 - 2.54)	2.43 (0.037) (2.37 - 2.46)	0.070 (0.046) (0.039 - 0.16)	-0.050, 0.19	0.193	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.51 (0.098) (14.39 - 14.63)	14.39 (0.11) (14.06 - 14.61)	0.12 (0.13) (0.00041 - 0.47)	-0.20, 0.45	0.374	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	56.07 (0.24) (55.90 - 56.24)	56.59 (0.28) (56.02 - 57.32)	-0.52 (0.35) (-1.16 - -0.12)	-1.41, 0.38	0.197	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.14 (0.0066) (0.11 - 0.15)	0.15 (0.0076) (0.14 - 0.15)	-0.0076 (0.010) (-0.0064 - 0.0089)	-0.033, 0.018	0.485	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.29 (0.0098) (0.26 - 0.30)	0.28 (0.011) (0.26 - 0.29)	0.015 (0.013) (0.0032 - 0.042)	-0.019, 0.049	0.309	0.17, 0.38 (0.20 - 0.36)

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.15 (0.0026) (0.14 - 0.16)	0.14 (0.0030) (0.14 - 0.15)	0.0075 (0.0037) (0.0012 - 0.013)	-0.0021, 0.017	0.100	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.13 (0.0015) (0.13 - 0.13)	0.11 (0.0018) (0.11 - 0.11)	0.020 (0.0023) (0.016 - 0.024)	0.014, 0.026	<0.001	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	8.67 (0.26) (8.13 - 9.20)	8.21 (0.30) (7.48 - 8.64)	0.46 (0.39) (0.055 - 1.72)	-0.54, 1.47	0.288	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	74.17 (3.80) (59.77 - 87.01)	78.00 (4.39) (75.01 - 80.40)	-3.83 (5.81) (-20.63 - -0.99)	-18.76, 11.10	0.539	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.41 (0.0052) (0.40 - 0.43)	0.38 (0.0054) (0.37 - 0.39)	0.032 (0.0034) (0.026 - 0.034)	0.023, 0.040	<0.001	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	14.26 (0.31) (13.82 - 15.04)	11.51 (0.34) (10.81 - 11.75)	2.75 (0.37) (2.07 - 3.10)	1.80, 3.70	<0.001	9.07, 17.33 (9.07 - 17.14)

Table E-25. Statistical Summary of Site GACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.78 (0.011) (0.75 - 0.81)	0.76 (0.012) (0.75 - 0.79)	0.012 (0.0082) (0.00079 - 0.021)	-0.0088, 0.034	0.192	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.22 (0.012) (1.18 - 1.24)	1.12 (0.013) (1.10 - 1.13)	0.098 (0.010) (0.087 - 0.11)	0.072, 0.12	<0.001	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.022 (0.0045) (0.0053 - 0.039)	0.017 (0.0052) (0.013 - 0.022)	0.0051 (0.0069) (-0.0019 - 0.026)	-0.013, 0.023	0.497	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	38.62 (0.67) (37.11 - 40.86)	39.55 (0.75) (38.49 - 40.84)	-0.93 (0.83) (-2.50 - 1.19)	-3.06, 1.20	0.311	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	149.29 (2.27) (142.84 - 153.55)	140.12 (2.63) (133.64 - 145.15)	9.17 (3.47) (-2.30 - 15.25)	0.24, 18.10	0.046	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-26. Statistical Summary of Site GACH Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.16 (0.0067) (0.14 - 0.17)	0.12 (0.0077) (0.11 - 0.13)	0.039 (0.010) (0.020 - 0.058)	0.012, 0.065	0.012	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.41 (0.027) (0.36 - 0.43)	0.32 (0.031) (0.31 - 0.34)	0.087 (0.038) (0.085 - 0.11)	-0.011, 0.19	0.072	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.011) (0.20 - 0.24)	0.18 (0.013) (0.18 - 0.20)	0.045 (0.015) (0.039 - 0.059)	0.0071, 0.082	0.028	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.91 (0.017) (0.85 - 0.95)	0.86 (0.019) (0.85 - 0.90)	0.044 (0.020) (0.036 - 0.091)	-0.0089, 0.096	0.085	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.98 (0.019) (0.93 - 1.03)	0.96 (0.020) (0.90 - 0.99)	0.024 (0.017) (-0.010 - 0.061)	-0.020, 0.068	0.221	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.46 (0.063) (4.40 - 4.55)	4.29 (0.071) (4.21 - 4.33)	0.16 (0.078) (0.071 - 0.23)	-0.038, 0.37	0.091	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	500.94 (2.75) (494.73 - 507.94)	495.83 (3.16) (492.30 - 504.10)	5.11 (3.87) (0.76 - 15.41)	-4.84, 15.06	0.244	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.42 (0.71) (42.57 - 46.25)	44.23 (0.82) (42.53 - 45.14)	0.19 (1.09) (-0.94 - 0.041)	-2.62, 2.99	0.870	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	7.08 (0.17) (6.51 - 7.47)	7.36 (0.19) (7.17 - 7.62)	-0.29 (0.20) (-0.85 - 0.13)	-0.81, 0.23	0.213	4.79, 9.92 (6.05 - 10.50)
Protein	27.36 (0.62) (26.05 - 28.45)	28.82 (0.72) (28.58 - 29.04)	-1.47 (0.93) (-2.53 - -0.58)	-3.86, 0.93	0.176	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.76 (0.55) (22.48 - 25.18)	22.62 (0.64) (21.87 - 24.18)	1.13 (0.81) (0.42 - 3.21)	-0.96, 3.22	0.222	15.01, 28.51 (16.58 - 25.25)

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	24.37 (0.55) (23.30 - 25.67)	24.01 (0.61) (22.08 - 25.22)	0.36 (0.65) (-0.26 - 1.22)	-1.31, 2.03	0.601	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	16.71 (0.24) (16.10 - 17.37)	17.67 (0.28) (17.49 - 17.88)	-0.96 (0.37) (-1.54 - -0.51)	-1.91, -0.015	0.047	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	28.65 (0.88) (28.05 - 29.04)	30.20 (1.01) (28.87 - 32.60)	-1.54 (1.21) (-3.72 - -0.82)	-4.66, 1.57	0.259	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	38.55 (0.42) (37.44 - 39.47)	40.14 (0.49) (39.32 - 41.35)	-1.59 (0.63) (-2.29 - -1.13)	-3.22, 0.044	0.054	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.03 (0.011) (1.02 - 1.04)	1.06 (0.012) (1.02 - 1.10)	-0.035 (0.014) (-0.061 - 0.0081)	-0.071, 0.00096	0.054	0.86, 1.11 (0.83 - 1.22)
Arginine	3.00 (0.053) (2.88 - 3.05)	3.28 (0.060) (3.10 - 3.43)	-0.28 (0.069) (-0.37 - -0.059)	-0.45, -0.10	0.009	2.38, 3.47 (2.30 - 3.55)

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.35 (0.037) (2.32 - 2.37)	2.50 (0.043) (2.39 - 2.64)	-0.15 (0.053) (-0.27 - -0.047)	-0.28, -0.012	0.038	1.94, 2.57 (1.79 - 2.72)
Cystine	0.39 (0.010) (0.36 - 0.42)	0.42 (0.012) (0.41 - 0.43)	-0.032 (0.016) (-0.074 - 0.011)	-0.073, 0.0083	0.096	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.68 (0.12) (4.63 - 4.71)	5.07 (0.14) (4.86 - 5.43)	-0.40 (0.18) (-0.72 - -0.18)	-0.86, 0.062	0.076	3.74, 5.28 (3.39 - 5.45)
Glycine	1.07 (0.018) (1.05 - 1.08)	1.11 (0.021) (1.07 - 1.18)	-0.047 (0.027) (-0.11 - 0.016)	-0.12, 0.023	0.144	0.90, 1.14 (0.85 - 1.23)
Histidine	0.73 (0.013) (0.69 - 0.76)	0.77 (0.014) (0.74 - 0.80)	-0.050 (0.011) (-0.069 - -0.031)	-0.078, -0.022	0.006	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.90 (0.014) (0.88 - 0.94)	0.95 (0.015) (0.91 - 0.98)	-0.049 (0.014) (-0.074 - -0.017)	-0.085, -0.013	0.017	0.75, 0.96 (0.72 - 1.03)

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.51 (0.020) (1.47 - 1.55)	1.58 (0.022) (1.52 - 1.65)	-0.074 (0.021) (-0.095 - -0.013)	-0.13, -0.020	0.017	1.25, 1.62 (1.20 - 1.72)
Lysine	1.19 (0.017) (1.16 - 1.22)	1.25 (0.019) (1.19 - 1.30)	-0.065 (0.021) (-0.084 - -0.024)	-0.12, -0.010	0.028	1.01, 1.30 (0.99 - 1.44)
Methionine	0.39 (0.015) (0.38 - 0.41)	0.39 (0.017) (0.34 - 0.44)	0.0063 (0.019) (-0.053 - 0.049)	-0.041, 0.054	0.747	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.42 (0.022) (1.37 - 1.45)	1.53 (0.025) (1.45 - 1.58)	-0.11 (0.029) (-0.14 - -0.013)	-0.18, -0.031	0.014	1.12, 1.58 (1.10 - 1.63)
Proline	1.00 (0.018) (0.96 - 1.03)	1.07 (0.020) (1.03 - 1.12)	-0.065 (0.027) (-0.094 - -0.00058)	-0.13, 0.0039	0.059	0.83, 1.08 (0.79 - 1.17)
Serine	1.07 (0.029) (1.05 - 1.07)	1.11 (0.034) (1.06 - 1.20)	-0.044 (0.045) (-0.14 - 0.017)	-0.16, 0.071	0.373	0.83, 1.21 (0.81 - 1.24)

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.84 (0.013) (0.82 - 0.86)	0.88 (0.015) (0.83 - 0.92)	-0.036 (0.019) (-0.062 - 0.021)	-0.086, 0.014	0.121	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.42 (0.0061) (0.40 - 0.44)	0.42 (0.0070) (0.42 - 0.43)	-0.0058 (0.0090) (-0.024 - 0.012)	-0.029, 0.017	0.551	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.79 (0.014) (0.76 - 0.80)	0.84 (0.016) (0.79 - 0.87)	-0.050 (0.019) (-0.076 - -0.0034)	-0.098, -0.0013	0.045	0.67, 0.84 (0.63 - 0.91)
Valine	1.20 (0.017) (1.15 - 1.23)	1.26 (0.019) (1.21 - 1.30)	-0.058 (0.023) (-0.076 - -0.0020)	-0.12, 0.00007	0.050	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.69 (0.0087) (0.66 - 0.70)	0.72 (0.0096) (0.71 - 0.73)	-0.033 (0.0090) (-0.050 - -0.020)	-0.056, -0.010	0.013	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	22.54 (0.089) (22.30 - 22.75)	22.73 (0.10) (22.69 - 22.78)	-0.19 (0.13) (-0.48 - 0.057)	-0.52, 0.15	0.208	16.54, 30.55 (19.11 - 26.73)

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.48 (0.0057) (0.47 - 0.50)	0.49 (0.0062) (0.49 - 0.50)	-0.0079 (0.0059) (-0.015 - 0.0026)	-0.023, 0.0073	0.239	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.25 (0.031) (2.16 - 2.34)	2.20 (0.036) (2.15 - 2.28)	0.048 (0.047) (-0.066 - 0.16)	-0.074, 0.17	0.356	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.65 (0.11) (14.23 - 14.99)	14.83 (0.13) (14.74 - 14.99)	-0.18 (0.17) (-0.54 - 0.0051)	-0.62, 0.27	0.356	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	58.08 (0.19) (57.39 - 58.48)	57.78 (0.22) (57.65 - 57.93)	0.30 (0.29) (-0.26 - 0.73)	-0.43, 1.04	0.335	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.18 (0.0027) (0.17 - 0.18)	0.17 (0.0030) (0.17 - 0.18)	0.0014 (0.0033) (-0.0031 - 0.0059)	-0.0070, 0.0099	0.684	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.23 (0.0029) (0.23 - 0.24)	0.24 (0.0034) (0.23 - 0.24)	-0.0038 (0.0045) (-0.011 - 0.0067)	-0.015, 0.0077	0.435	0.17, 0.38 (0.20 - 0.36)

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.13 (0.0030) (0.12 - 0.14)	0.13 (0.0035) (0.13 - 0.14)	-0.0068 (0.0046) (-0.019 - 0.0086)	-0.019, 0.0049	0.196	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.20 (0.0060) (0.18 - 0.21)	0.18 (0.0065) (0.17 - 0.19)	0.018 (0.0061) (0.0037 - 0.021)	0.0022, 0.034	0.032	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	10.86 (0.54) (9.15 - 12.15)	11.01 (0.61) (10.09 - 11.33)	-0.15 (0.71) (-2.19 - 1.26)	-1.97, 1.67	0.842	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	90.85 (5.50) (74.66 - 109.70)	74.39 (6.35) (72.65 - 76.27)	16.46 (8.40) (1.19 - 27.33)	-5.13, 38.05	0.107	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.42 (0.0033) (0.41 - 0.43)	0.40 (0.0038) (0.39 - 0.40)	0.023 (0.0046) (0.015 - 0.026)	0.011, 0.035	0.004	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	14.18 (0.29) (13.48 - 14.84)	12.56 (0.33) (11.96 - 13.28)	1.62 (0.44) (0.20 - 2.14)	0.50, 2.74	0.013	9.07, 17.33 (9.07 - 17.14)

Table E-27. Statistical Summary of Site KSLA Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.76 (0.0090) (0.75 - 0.78)	0.78 (0.010) (0.77 - 0.79)	-0.014 (0.011) (-0.020 - -0.0075)	-0.042, 0.014	0.251	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.11 (0.010) (1.08 - 1.12)	1.08 (0.012) (1.05 - 1.10)	0.029 (0.015) (0.020 - 0.030)	-0.0087, 0.067	0.104	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.015 (0.0028) (0.0054 - 0.022)	0.0080 (0.0032) (0.0054 - 0.013)	0.0070 (0.0042) (0.0062 - 0.016)	-0.0039, 0.018	0.159	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	40.63 (1.18) (36.90 - 42.39)	42.00 (1.37) (40.59 - 43.50)	-1.37 (1.81) (-6.60 - 0.49)	-6.01, 3.27	0.482	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	94.05 (2.67) (87.22 - 99.35)	92.34 (2.91) (91.78 - 95.85)	1.71 (2.71) (-2.10 - 7.57)	-5.26, 8.67	0.556	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-28. Statistical Summary of Site KSLA Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.14 (0.010) (0.13 - 0.15)	0.12 (0.012) (0.12 - 0.13)	0.017 (0.014) (0.00032 - 0.031)	-0.020, 0.054	0.296	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.41 (0.030) (0.35 - 0.45)	0.37 (0.034) (0.33 - 0.39)	0.044 (0.039) (-0.012 - 0.094)	-0.056, 0.15	0.308	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.21 (0.014) (0.19 - 0.22)	0.20 (0.016) (0.19 - 0.21)	0.012 (0.019) (-0.019 - 0.030)	-0.036, 0.060	0.552	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	1.00 (0.027) (0.96 - 1.03)	0.95 (0.030) (0.86 - 1.05)	0.055 (0.033) (-0.030 - 0.13)	-0.029, 0.14	0.151	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.09 (0.033) (1.05 - 1.12)	1.01 (0.038) (1.00 - 1.02)	0.075 (0.047) (0.073 - 0.11)	-0.046, 0.20	0.172	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.27 (0.047) (4.14 - 4.40)	4.12 (0.047) (4.06 - 4.15)	0.16 (0.066) (0.081 - 0.25)	-0.0069, 0.32	0.057	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g dw)	498.08 (3.01) (493.35 - 504.84)	494.75 (3.01) (490.27 - 498.67)	3.33 (4.19) (-3.41 - 14.57)	-6.92, 13.58	0.457	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	45.34 (0.79) (44.46 - 46.08)	47.84 (0.79) (46.77 - 50.30)	-2.50 (1.11) (-5.09 - -1.23)	-5.22, 0.23	0.066	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.44 (0.26) (5.81 - 7.10)	6.98 (0.26) (6.15 - 7.40)	-0.54 (0.36) (-1.45 - 0.11)	-1.43, 0.35	0.190	4.79, 9.92 (6.05 - 10.50)
Protein	27.41 (0.56) (26.70 - 28.07)	25.80 (0.56) (23.53 - 27.85)	1.62 (0.80) (-1.15 - 4.39)	-0.33, 3.56	0.088	22.30, 29.41 (20.58 - 29.28)
Total Fat	22.96 (0.64) (22.01 - 24.43)	22.25 (0.64) (21.29 - 23.02)	0.71 (0.90) (-0.67 - 3.15)	-1.49, 2.90	0.462	15.01, 28.51 (16.58 - 25.25)

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.94 (0.45) (25.56 - 26.33)	28.35 (0.45) (27.81 - 29.58)	-2.41 (0.63) (-4.02 - -1.48)	-3.96, -0.86	0.008	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.47 (0.66) (14.96 - 19.14)	19.62 (0.66) (18.46 - 20.54)	-2.15 (0.93) (-5.57 - 0.12)	-4.42, 0.12	0.059	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	32.12 (0.65) (30.20 - 33.94)	34.05 (0.65) (32.61 - 35.84)	-1.93 (0.42) (-2.42 - -1.25)	-2.96, -0.89	0.003	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	41.09 (0.49) (39.95 - 42.26)	43.35 (0.49) (42.33 - 44.37)	-2.26 (0.69) (-4.41 - -0.26)	-3.94, -0.58	0.016	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.06 (0.018) (1.02 - 1.07)	1.03 (0.018) (1.00 - 1.06)	0.028 (0.014) (0.012 - 0.047)	-0.0052, 0.062	0.084	0.86, 1.11 (0.83 - 1.22)
Arginine	3.00 (0.073) (2.92 - 3.09)	2.98 (0.073) (2.89 - 3.13)	0.018 (0.084) (-0.13 - 0.16)	-0.19, 0.22	0.836	2.38, 3.47 (2.30 - 3.55)

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.38 (0.050) (2.35 - 2.45)	2.30 (0.050) (2.25 - 2.40)	0.075 (0.062) (-0.040 - 0.18)	-0.076, 0.23	0.269	1.94, 2.57 (1.79 - 2.72)
Cystine	0.38 (0.016) (0.35 - 0.40)	0.38 (0.016) (0.36 - 0.44)	-0.0067 (0.019) (-0.038 - 0.031)	-0.054, 0.041	0.741	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.67 (0.13) (4.57 - 4.83)	4.52 (0.13) (4.37 - 4.75)	0.15 (0.16) (-0.18 - 0.36)	-0.25, 0.55	0.384	3.74, 5.28 (3.39 - 5.45)
Glycine	1.10 (0.020) (1.07 - 1.12)	1.06 (0.020) (1.04 - 1.09)	0.039 (0.025) (0.012 - 0.076)	-0.022, 0.099	0.168	0.90, 1.14 (0.85 - 1.23)
Histidine	0.73 (0.020) (0.68 - 0.77)	0.72 (0.020) (0.67 - 0.76)	0.010 (0.014) (-0.015 - 0.062)	-0.023, 0.044	0.477	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.91 (0.018) (0.88 - 0.93)	0.88 (0.018) (0.87 - 0.90)	0.029 (0.020) (0.017 - 0.056)	-0.019, 0.077	0.186	0.75, 0.96 (0.72 - 1.03)

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.52 (0.026) (1.50 - 1.54)	1.48 (0.026) (1.44 - 1.52)	0.045 (0.028) (0.013 - 0.079)	-0.024, 0.11	0.158	1.25, 1.62 (1.20 - 1.72)
Lysine	1.25 (0.024) (1.23 - 1.27)	1.18 (0.024) (1.12 - 1.23)	0.074 (0.027) (0.023 - 0.14)	0.0069, 0.14	0.035	1.01, 1.30 (0.99 - 1.44)
Methionine	0.40 (0.017) (0.38 - 0.42)	0.38 (0.017) (0.32 - 0.42)	0.028 (0.013) (-0.0027 - 0.064)	-0.0036, 0.060	0.073	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.43 (0.027) (1.41 - 1.46)	1.39 (0.027) (1.36 - 1.43)	0.038 (0.033) (-0.0091 - 0.090)	-0.043, 0.12	0.296	1.12, 1.58 (1.10 - 1.63)
Proline	1.03 (0.015) (1.02 - 1.05)	0.98 (0.015) (0.95 - 1.02)	0.045 (0.022) (0.0012 - 0.10)	-0.0079, 0.099	0.082	0.83, 1.08 (0.79 - 1.17)
Serine	1.06 (0.022) (1.04 - 1.08)	1.03 (0.022) (1.01 - 1.06)	0.025 (0.030) (0.0023 - 0.049)	-0.049, 0.099	0.445	0.83, 1.21 (0.81 - 1.24)

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.87 (0.016) (0.83 - 0.89)	0.84 (0.016) (0.82 - 0.86)	0.023 (0.012) (0.0022 - 0.034)	-0.0073, 0.053	0.112	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.40 (0.014) (0.39 - 0.43)	0.39 (0.014) (0.38 - 0.43)	0.0098 (0.018) (-0.033 - 0.041)	-0.035, 0.054	0.610	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.81 (0.018) (0.81 - 0.82)	0.79 (0.018) (0.76 - 0.81)	0.023 (0.021) (-0.0051 - 0.050)	-0.029, 0.075	0.320	0.67, 0.84 (0.63 - 0.91)
Valine	1.23 (0.024) (1.20 - 1.26)	1.18 (0.024) (1.17 - 1.19)	0.047 (0.029) (0.0063 - 0.080)	-0.023, 0.12	0.152	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.73 (0.013) (0.69 - 0.77)	0.75 (0.013) (0.73 - 0.78)	-0.020 (0.018) (-0.087 - 0.041)	-0.065, 0.024	0.312	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.26 (0.091) (23.95 - 24.55)	24.04 (0.091) (23.92 - 24.16)	0.21 (0.13) (-0.13 - 0.63)	-0.099, 0.53	0.145	16.54, 30.55 (19.11 - 26.73)

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.50 (0.0035) (0.50 - 0.51)	0.50 (0.0035) (0.49 - 0.51)	0.00039 (0.0049) (-0.011 - 0.012)	-0.012, 0.012	0.939	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.61 (0.018) (2.58 - 2.64)	2.52 (0.018) (2.49 - 2.57)	0.089 (0.026) (0.026 - 0.15)	0.025, 0.15	0.014	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.46 (0.095) (14.26 - 14.65)	14.29 (0.095) (14.13 - 14.53)	0.17 (0.13) (-0.27 - 0.52)	-0.16, 0.50	0.252	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	56.04 (0.14) (55.71 - 56.35)	56.63 (0.14) (56.52 - 56.72)	-0.58 (0.20) (-0.91 - -0.30)	-1.08, -0.092	0.027	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.15 (0.0042) (0.14 - 0.16)	0.15 (0.0042) (0.13 - 0.15)	0.0015 (0.0059) (-0.012 - 0.014)	-0.013, 0.016	0.802	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.29 (0.0054) (0.27 - 0.31)	0.29 (0.0054) (0.29 - 0.30)	0.0028 (0.0071) (-0.022 - 0.024)	-0.015, 0.020	0.710	0.17, 0.38 (0.20 - 0.36)

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.15 (0.0039) (0.13 - 0.16)	0.14 (0.0039) (0.14 - 0.15)	0.0070 (0.0055) (-0.0068 - 0.027)	-0.0064, 0.020	0.247	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.13 (0.0039) (0.12 - 0.14)	0.12 (0.0039) (0.12 - 0.12)	0.0092 (0.0050) (0.0045 - 0.017)	-0.0030, 0.021	0.115	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	8.24 (0.30) (7.69 - 8.79)	8.70 (0.30) (8.11 - 9.14)	-0.47 (0.42) (-1.27 - 0.086)	-1.51, 0.57	0.311	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	77.97 (4.19) (69.22 - 98.27)	68.59 (4.19) (66.28 - 70.38)	9.38 (4.72) (-0.024 - 27.89)	-2.16, 20.93	0.093	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.41 (0.0065) (0.41 - 0.42)	0.39 (0.0065) (0.38 - 0.41)	0.020 (0.0093) (0.0091 - 0.029)	-0.0022, 0.043	0.068	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	13.87 (0.37) (13.02 - 14.71)	12.87 (0.37) (12.31 - 13.87)	0.99 (0.52) (-0.84 - 2.08)	-0.29, 2.28	0.106	9.07, 17.33 (9.07 - 17.14)

Table E-29. Statistical Summary of Site LACH Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.72 (0.014) (0.68 - 0.73)	0.71 (0.014) (0.69 - 0.73)	0.0034 (0.019) (-0.011 - 0.021)	-0.044, 0.051	0.867	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.15 (0.022) (1.13 - 1.18)	1.17 (0.022) (1.12 - 1.27)	-0.023 (0.029) (-0.13 - 0.026)	-0.093, 0.048	0.461	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.023 (0.0043) (0.0054 - 0.029)	0.015 (0.0043) (0.0053 - 0.027)	0.0074 (0.0060) (-0.0081 - 0.024)	-0.0072, 0.022	0.261	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	34.21 (0.93) (32.91 - 35.63)	35.74 (0.93) (35.10 - 37.09)	-1.54 (1.32) (-2.57 - -1.04)	-4.75, 1.68	0.287	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	167.00 (2.48) (158.95 - 173.30)	149.96 (2.48) (148.96 - 152.67)	17.04 (3.48) (9.99 - 20.70)	8.52, 25.55	0.002	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]Test refers to MON 88701 (Not Treated). These plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-30. Statistical Summary of Site LACH Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.16 (0.011) (0.14 - 0.19)	0.13 (0.011) (0.12 - 0.14)	0.030 (0.016) (-0.0041 - 0.055)	-0.0078, 0.069	0.099	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.41 (0.035) (0.36 - 0.50)	0.35 (0.035) (0.31 - 0.38)	0.059 (0.049) (0.011 - 0.19)	-0.062, 0.18	0.278	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.016) (0.21 - 0.27)	0.20 (0.016) (0.17 - 0.21)	0.029 (0.023) (-0.0031 - 0.093)	-0.028, 0.085	0.260	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.84 (0.026) (0.76 - 0.90)	0.81 (0.026) (0.78 - 0.84)	0.031 (0.036) (-0.046 - 0.12)	-0.057, 0.12	0.420	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.93 (0.025) (0.89 - 1.01)	0.90 (0.025) (0.82 - 0.94)	0.035 (0.036) (-0.028 - 0.18)	-0.052, 0.12	0.363	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.33 (0.057) (4.20 - 4.48)	4.14 (0.064) (3.94 - 4.26)	0.19 (0.070) (0.038 - 0.36)	0.0083, 0.37	0.043	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	498.21 (1.61) (496.22 - 499.62)	491.80 (1.86) (488.93 - 494.48)	6.41 (2.46) (3.24 - 10.35)	0.078, 12.75	0.048	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	43.25 (0.59) (41.07 - 44.88)	44.36 (0.68) (43.65 - 45.15)	-1.11 (0.87) (-3.08 - 1.23)	-3.36, 1.13	0.259	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	8.67 (0.22) (7.98 - 9.07)	8.96 (0.23) (8.59 - 9.19)	-0.29 (0.19) (-0.61 - -0.010)	-0.76, 0.19	0.184	4.79, 9.92 (6.05 - 10.50)
Protein	29.35 (0.61) (27.82 - 31.29)	29.84 (0.70) (29.62 - 30.42)	-0.49 (0.85) (-2.05 - 0.88)	-2.68, 1.70	0.592	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.08 (0.30) (22.78 - 23.39)	21.59 (0.34) (21.03 - 22.21)	1.49 (0.46) (0.72 - 2.17)	0.32, 2.66	0.022	15.01, 28.51 (16.58 - 25.25)

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	26.21 (1.12) (23.72 - 30.43)	26.31 (1.29) (24.72 - 28.08)	-0.10 (1.71) (-2.57 - 5.70)	-4.50, 4.30	0.955	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.82 (0.29) (17.57 - 18.04)	16.93 (0.33) (16.30 - 17.90)	0.90 (0.38) (-0.33 - 1.52)	-0.094, 1.88	0.067	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	32.76 (1.03) (31.18 - 37.27)	31.14 (1.19) (30.85 - 31.49)	1.61 (1.58) (-0.26 - 6.42)	-2.44, 5.67	0.353	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	38.49 (0.55) (37.06 - 40.31)	39.52 (0.64) (39.05 - 39.86)	-1.03 (0.85) (-2.58 - -1.10)	-3.21, 1.14	0.277	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.13 (0.020) (1.06 - 1.15)	1.10 (0.023) (1.05 - 1.14)	0.027 (0.031) (-0.075 - 0.11)	-0.052, 0.11	0.418	0.86, 1.11 (0.83 - 1.22)
Arginine	3.10 (0.052) (2.92 - 3.22)	3.20 (0.060) (3.11 - 3.27)	-0.10 (0.080) (-0.30 - 0.011)	-0.31, 0.10	0.253	2.38, 3.47 (2.30 - 3.55)

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.56 (0.053) (2.35 - 2.67)	2.55 (0.061) (2.46 - 2.63)	0.013 (0.081) (-0.28 - 0.14)	-0.19, 0.22	0.876	1.94, 2.57 (1.79 - 2.72)
Cystine	0.42 (0.018) (0.38 - 0.49)	0.42 (0.021) (0.39 - 0.46)	0.0075 (0.028) (-0.073 - 0.086)	-0.064, 0.079	0.798	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.90 (0.13) (4.39 - 5.18)	5.08 (0.15) (4.85 - 5.40)	-0.18 (0.20) (-1.01 - 0.18)	-0.69, 0.33	0.405	3.74, 5.28 (3.39 - 5.45)
Glycine	1.13 (0.019) (1.07 - 1.17)	1.11 (0.022) (1.08 - 1.14)	0.020 (0.029) (-0.063 - 0.062)	-0.054, 0.093	0.522	0.90, 1.14 (0.85 - 1.23)
Histidine	0.77 (0.013) (0.75 - 0.80)	0.76 (0.015) (0.74 - 0.79)	0.015 (0.019) (0.0060 - 0.024)	-0.034, 0.064	0.466	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.95 (0.018) (0.91 - 1.00)	0.96 (0.020) (0.93 - 0.97)	-0.0094 (0.020) (-0.028 - 0.022)	-0.061, 0.043	0.661	0.75, 0.96 (0.72 - 1.03)

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.63 (0.023) (1.56 - 1.68)	1.60 (0.027) (1.55 - 1.63)	0.024 (0.035) (-0.065 - 0.088)	-0.067, 0.11	0.533	1.25, 1.62 (1.20 - 1.72)
Lysine	1.32 (0.029) (1.27 - 1.37)	1.26 (0.033) (1.22 - 1.29)	0.057 (0.043) (-0.0085 - 0.086)	-0.054, 0.17	0.243	1.01, 1.30 (0.99 - 1.44)
Methionine	0.42 (0.0093) (0.39 - 0.44)	0.41 (0.011) (0.40 - 0.42)	0.0089 (0.014) (-0.0049 - 0.019)	-0.028, 0.046	0.557	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.49 (0.027) (1.42 - 1.55)	1.52 (0.031) (1.45 - 1.56)	-0.026 (0.041) (-0.14 - 0.055)	-0.13, 0.079	0.548	1.12, 1.58 (1.10 - 1.63)
Proline	1.08 (0.022) (1.06 - 1.13)	1.09 (0.025) (1.07 - 1.12)	-0.013 (0.029) (-0.057 - 0.032)	-0.088, 0.063	0.684	0.83, 1.08 (0.79 - 1.17)
Serine	1.16 (0.027) (1.05 - 1.22)	1.13 (0.032) (1.09 - 1.19)	0.032 (0.042) (-0.14 - 0.13)	-0.076, 0.14	0.483	0.83, 1.21 (0.81 - 1.24)

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.92 (0.014) (0.87 - 0.95)	0.89 (0.016) (0.86 - 0.92)	0.029 (0.021) (-0.050 - 0.079)	-0.026, 0.083	0.237	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.44 (0.021) (0.41 - 0.50)	0.45 (0.024) (0.39 - 0.52)	-0.016 (0.032) (-0.099 - 0.11)	-0.099, 0.067	0.642	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.85 (0.012) (0.83 - 0.87)	0.84 (0.014) (0.82 - 0.87)	0.0095 (0.018) (-0.0099 - 0.043)	-0.036, 0.055	0.618	0.67, 0.84 (0.63 - 0.91)
Valine	1.26 (0.025) (1.21 - 1.32)	1.30 (0.027) (1.24 - 1.32)	-0.036 (0.021) (-0.074 - -0.0017)	-0.090, 0.017	0.140	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.71 (0.0074) (0.69 - 0.73)	0.75 (0.0086) (0.74 - 0.76)	-0.036 (0.011) (-0.065 - -0.013)	-0.065, -0.0069	0.024	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	23.40 (0.12) (23.11 - 23.69)	23.10 (0.14) (23.07 - 23.15)	0.30 (0.18) (0.26 - 0.62)	-0.16, 0.76	0.152	16.54, 30.55 (19.11 - 26.73)

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.47 (0.0047) (0.46 - 0.48)	0.48 (0.0053) (0.47 - 0.49)	-0.0094 (0.0055) (-0.024 - 0.0016)	-0.023, 0.0047	0.147	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.53 (0.037) (2.49 - 2.57)	2.34 (0.043) (2.32 - 2.38)	0.19 (0.057) (0.11 - 0.26)	0.041, 0.33	0.021	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	15.29 (0.12) (15.01 - 15.50)	14.70 (0.14) (14.51 - 14.83)	0.59 (0.19) (0.18 - 1.00)	0.11, 1.08	0.026	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	56.04 (0.23) (55.76 - 56.31)	57.19 (0.26) (57.01 - 57.46)	-1.15 (0.35) (-1.65 - -0.71)	-2.04, -0.25	0.021	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.36 (0.012) (0.34 - 0.38)	0.29 (0.014) (0.27 - 0.30)	0.074 (0.018) (0.048 - 0.11)	0.028, 0.12	0.008	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.30 (0.0065) (0.29 - 0.30)	0.28 (0.0075) (0.27 - 0.28)	0.023 (0.0099) (0.013 - 0.031)	-0.0021, 0.049	0.065	0.17, 0.38 (0.20 - 0.36)

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.16 (0.0056) (0.16 - 0.17)	0.15 (0.0065) (0.15 - 0.16)	0.0093 (0.0086) (-0.00007 - 0.015)	-0.013, 0.031	0.327	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0014) (0.15 - 0.15)	0.14 (0.0017) (0.14 - 0.14)	0.013 (0.0022) (0.0067 - 0.018)	0.0072, 0.018	0.002	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	6.59 (0.36) (5.37 - 7.35)	6.91 (0.41) (6.64 - 7.19)	-0.31 (0.54) (-0.067 - 0.19)	-1.71, 1.08	0.587	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	44.35 (1.00) (41.73 - 46.45)	48.04 (1.15) (45.03 - 50.87)	-3.69 (1.53) (-9.14 - -0.44)	-7.62, 0.23	0.060	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.41 (0.010) (0.40 - 0.42)	0.40 (0.012) (0.37 - 0.44)	0.010 (0.016) (-0.015 - 0.055)	-0.031, 0.051	0.545	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	14.78 (0.36) (13.36 - 15.70)	13.83 (0.42) (13.65 - 14.11)	0.95 (0.56) (0.97 - 1.97)	-0.48, 2.38	0.148	9.07, 17.33 (9.07 - 17.14)

Table E-31. Statistical Summary of Site NCBD Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.64 (0.020) (0.60 - 0.66)	0.66 (0.023) (0.59 - 0.71)	-0.021 (0.031) (-0.078 - 0.072)	-0.10, 0.058	0.521	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.04 (0.019) (1.03 - 1.06)	1.08 (0.022) (1.03 - 1.16)	-0.045 (0.029) (-0.13 - -0.0056)	-0.12, 0.030	0.183	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.074 (0.0084) (0.061 - 0.082)	0.099 (0.0096) (0.094 - 0.10)	-0.026 (0.011) (-0.038 - -0.020)	-0.055, 0.0031	0.069	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	41.50 (1.30) (39.45 - 43.05)	49.54 (1.50) (44.04 - 52.95)	-8.04 (1.98) (-13.50 - -2.09)	-13.13, -2.95	0.009	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	174.64 (3.66) (165.41 - 184.47)	156.99 (4.23) (151.55 - 162.98)	17.65 (5.60) (7.48 - 32.93)	3.27, 32.04	0.025	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-32. Statistical Summary of Site NCBD Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0093) (0.12 - 0.18)	0.13 (0.011) (0.12 - 0.14)	0.023 (0.014) (-0.0060 - 0.032)	-0.013, 0.059	0.167	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.36 (0.040) (0.26 - 0.45)	0.37 (0.046) (0.36 - 0.41)	-0.018 (0.056) (-0.11 - 0.039)	-0.16, 0.13	0.760	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.024) (0.16 - 0.27)	0.22 (0.028) (0.21 - 0.23)	0.010 (0.037) (-0.055 - 0.044)	-0.085, 0.11	0.791	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.94 (0.026) (0.88 - 0.99)	0.90 (0.030) (0.81 - 0.95)	0.044 (0.039) (-0.00010 - 0.18)	-0.057, 0.14	0.314	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.21 (0.067) (1.05 - 1.52)	1.09 (0.077) (1.08 - 1.10)	0.12 (0.10) (0.021 - 0.44)	-0.14, 0.38	0.287	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.31 (0.087) (4.25 - 4.38)	4.27 (0.10) (4.20 - 4.39)	0.042 (0.13) (-0.0052 - 0.087)	-0.29, 0.38	0.761	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	495.87 (2.64) (487.62 - 499.09)	492.81 (3.05) (490.52 - 494.31)	3.06 (4.04) (-5.97 - 7.23)	-7.32, 13.45	0.482	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	42.30 (0.46) (41.59 - 43.70)	42.60 (0.53) (42.14 - 43.05)	-0.30 (0.70) (-0.90 - 1.56)	-2.10, 1.50	0.688	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	7.03 (0.25) (6.78 - 7.42)	7.28 (0.28) (6.63 - 7.75)	-0.25 (0.32) (-0.92 - 0.15)	-1.08, 0.58	0.471	4.79, 9.92 (6.05 - 10.50)
Protein	30.79 (0.13) (30.68 - 30.89)	31.18 (0.15) (31.00 - 31.27)	-0.39 (0.19) (-0.59 - -0.31)	-0.89, 0.11	0.103	22.30, 29.41 (20.58 - 29.28)
Total Fat	22.62 (0.54) (20.99 - 23.29)	21.95 (0.62) (21.42 - 22.22)	0.67 (0.83) (-1.21 - 1.54)	-1.46, 2.79	0.455	15.01, 28.51 (16.58 - 25.25)

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	26.43 (0.51) (25.17 - 27.78)	26.40 (0.59) (25.76 - 27.10)	0.030 (0.79) (-0.16 - 1.44)	-1.99, 2.05	0.971	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.75 (0.74) (16.63 - 18.67)	17.71 (0.84) (16.06 - 20.78)	0.040 (0.94) (-2.11 - 2.09)	-2.39, 2.47	0.967	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	30.87 (0.87) (29.16 - 32.29)	32.83 (1.00) (31.58 - 34.49)	-1.96 (1.32) (-4.66 - 0.72)	-5.36, 1.44	0.199	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	38.30 (0.71) (37.12 - 39.32)	41.10 (0.75) (39.09 - 43.00)	-2.81 (0.55) (-4.03 - -1.98)	-4.22, -1.39	0.003	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.11 (0.011) (1.09 - 1.13)	1.13 (0.013) (1.09 - 1.17)	-0.019 (0.014) (-0.044 - 0.023)	-0.054, 0.015	0.211	0.86, 1.11 (0.83 - 1.22)
Arginine	3.51 (0.049) (3.34 - 3.62)	3.71 (0.053) (3.67 - 3.77)	-0.20 (0.048) (-0.23 - -0.12)	-0.32, -0.075	0.008	2.38, 3.47 (2.30 - 3.55)

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.63 (0.033) (2.52 - 2.69)	2.66 (0.037) (2.58 - 2.74)	-0.033 (0.043) (-0.091 - 0.069)	-0.14, 0.076	0.469	1.94, 2.57 (1.79 - 2.72)
Cystine	0.43 (0.013) (0.41 - 0.46)	0.44 (0.015) (0.43 - 0.45)	-0.0077 (0.020) (-0.022 - 0.0095)	-0.058, 0.043	0.713	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	5.31 (0.087) (5.08 - 5.57)	5.46 (0.10) (5.29 - 5.70)	-0.15 (0.13) (-0.50 - 0.18)	-0.49, 0.19	0.309	3.74, 5.28 (3.39 - 5.45)
Glycine	1.16 (0.014) (1.11 - 1.19)	1.18 (0.015) (1.17 - 1.20)	-0.018 (0.016) (-0.023 - 0.0094)	-0.060, 0.023	0.309	0.90, 1.14 (0.85 - 1.23)
Histidine	0.82 (0.010) (0.79 - 0.84)	0.83 (0.012) (0.83 - 0.84)	-0.010 (0.015) (-0.026 - 0.014)	-0.048, 0.027	0.510	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.97 (0.017) (0.94 - 1.01)	0.98 (0.018) (0.94 - 1.03)	-0.0018 (0.012) (-0.014 - 0.017)	-0.032, 0.028	0.885	0.75, 0.96 (0.72 - 1.03)

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.64 (0.021) (1.57 - 1.67)	1.66 (0.023) (1.63 - 1.69)	-0.015 (0.022) (-0.024 - 0.035)	-0.071, 0.041	0.519	1.25, 1.62 (1.20 - 1.72)
Lysine	1.31 (0.019) (1.29 - 1.33)	1.36 (0.022) (1.32 - 1.39)	-0.045 (0.022) (-0.057 - -0.0086)	-0.10, 0.012	0.097	1.01, 1.30 (0.99 - 1.44)
Methionine	0.42 (0.012) (0.39 - 0.44)	0.40 (0.014) (0.38 - 0.41)	0.021 (0.018) (-0.016 - 0.048)	-0.026, 0.069	0.295	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.58 (0.026) (1.50 - 1.63)	1.61 (0.028) (1.60 - 1.66)	-0.026 (0.022) (-0.034 - 0.013)	-0.083, 0.030	0.287	1.12, 1.58 (1.10 - 1.63)
Proline	1.12 (0.027) (1.07 - 1.16)	1.18 (0.031) (1.10 - 1.25)	-0.057 (0.039) (-0.13 - 0.055)	-0.16, 0.043	0.200	0.83, 1.08 (0.79 - 1.17)
Serine	1.20 (0.026) (1.11 - 1.28)	1.20 (0.030) (1.16 - 1.24)	-0.0068 (0.040) (-0.055 - 0.063)	-0.11, 0.097	0.872	0.83, 1.21 (0.81 - 1.24)

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.92 (0.015) (0.86 - 0.95)	0.93 (0.017) (0.91 - 0.94)	-0.0029 (0.021) (-0.00072 - 0.037)	-0.058, 0.052	0.896	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.44 (0.014) (0.43 - 0.44)	0.44 (0.016) (0.43 - 0.45)	-0.0052 (0.020) (-0.020 - 0.0087)	-0.057, 0.046	0.803	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.87 (0.014) (0.84 - 0.88)	0.89 (0.015) (0.86 - 0.91)	-0.018 (0.014) (-0.029 - 0.011)	-0.054, 0.018	0.250	0.67, 0.84 (0.63 - 0.91)
Valine	1.29 (0.024) (1.25 - 1.32)	1.34 (0.026) (1.31 - 1.40)	-0.044 (0.019) (-0.078 - -0.019)	-0.093, 0.0056	0.071	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.95 (0.0046) (0.94 - 0.95)	0.98 (0.0054) (0.97 - 0.98)	-0.030 (0.0071) (-0.032 - -0.026)	-0.048, -0.012	0.008	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.15 (0.088) (24.06 - 24.37)	24.11 (0.10) (23.89 - 24.34)	0.044 (0.13) (-0.23 - 0.47)	-0.30, 0.39	0.757	16.54, 30.55 (19.11 - 26.73)

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.53 (0.0022) (0.53 - 0.53)	0.54 (0.0025) (0.53 - 0.54)	-0.0080 (0.0033) (-0.014 - -0.0036)	-0.017, 0.00054	0.060	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.52 (0.020) (2.49 - 2.56)	2.64 (0.021) (2.61 - 2.70)	-0.12 (0.016) (-0.14 - -0.12)	-0.17, -0.082	<0.001	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	16.13 (0.067) (16.01 - 16.29)	16.21 (0.076) (16.10 - 16.35)	-0.085 (0.088) (-0.23 - -0.072)	-0.31, 0.14	0.382	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	54.43 (0.084) (54.22 - 54.63)	54.29 (0.097) (54.04 - 54.50)	0.14 (0.13) (-0.11 - 0.59)	-0.19, 0.47	0.326	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.16 (0.0019) (0.15 - 0.16)	0.14 (0.0022) (0.14 - 0.15)	0.014 (0.0029) (0.0032 - 0.020)	0.0065, 0.021	0.004	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.30 (0.0052) (0.29 - 0.31)	0.31 (0.0060) (0.28 - 0.32)	-0.0075 (0.0080) (-0.026 - 0.022)	-0.028, 0.013	0.389	0.17, 0.38 (0.20 - 0.36)

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.17 (0.0065) (0.16 - 0.17)	0.19 (0.0075) (0.17 - 0.21)	-0.020 (0.0099) (-0.057 - -0.00078)	-0.046, 0.0053	0.097	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.15 (0.0029) (0.14 - 0.15)	0.13 (0.0034) (0.12 - 0.13)	0.019 (0.0042) (0.015 - 0.024)	0.0082, 0.030	0.006	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	11.38 (0.15) (11.09 - 11.56)	11.75 (0.17) (11.46 - 11.92)	-0.37 (0.22) (-0.77 - 0.018)	-0.95, 0.20	0.157	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	67.95 (3.33) (61.15 - 83.28)	64.62 (3.84) (63.58 - 66.45)	3.33 (5.08) (-2.69 - -1.19)	-9.74, 16.39	0.541	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.38 (0.0053) (0.37 - 0.39)	0.37 (0.0061) (0.36 - 0.38)	0.0076 (0.0081) (-0.0069 - 0.021)	-0.013, 0.028	0.386	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	13.23 (0.23) (12.92 - 13.61)	12.90 (0.26) (12.00 - 13.47)	0.33 (0.34) (-0.55 - 1.20)	-0.55, 1.22	0.377	9.07, 17.33 (9.07 - 17.14)

Table E-33. Statistical Summary of Site NMLC Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.76 (0.010) (0.75 - 0.78)	0.79 (0.012) (0.78 - 0.80)	-0.031 (0.016) (-0.046 - -0.0099)	-0.071, 0.0099	0.109	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.11 (0.016) (1.09 - 1.12)	1.11 (0.018) (1.07 - 1.14)	-0.00071 (0.017) (-0.044 - 0.029)	-0.045, 0.043	0.968	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.017 (0.0034) (0.0054 - 0.023)	0.013 (0.0039) (0.0054 - 0.023)	0.0041 (0.0052) (-0.0019 - 0.018)	-0.0092, 0.017	0.465	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	44.99 (0.60) (44.56 - 46.04)	49.43 (0.69) (47.66 - 50.87)	-4.44 (0.92) (-6.30 - -3.03)	-6.80, -2.08	0.004	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	115.90 (2.04) (113.71 - 119.14)	112.18 (2.36) (107.02 - 115.99)	3.72 (3.12) (0.18 - 7.09)	-4.29, 11.73	0.285	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-34. Statistical Summary of Site NMLC Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.16 (0.0079) (0.14 - 0.18)	0.14 (0.0092) (0.12 - 0.15)	0.022 (0.012) (-0.0016 - 0.056)	-0.0097, 0.053	0.136	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.33 (0.023) (0.24 - 0.38)	0.29 (0.026) (0.26 - 0.31)	0.039 (0.035) (-0.070 - 0.13)	-0.050, 0.13	0.308	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.19 (0.018) (0.12 - 0.24)	0.18 (0.020) (0.17 - 0.19)	0.0079 (0.027) (-0.075 - 0.079)	-0.061, 0.077	0.780	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.86 (0.026) (0.76 - 0.95)	0.69 (0.030) (0.68 - 0.70)	0.17 (0.040) (0.12 - 0.27)	0.063, 0.27	0.008	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	0.88 (0.026) (0.84 - 0.92)	0.80 (0.030) (0.74 - 0.87)	0.083 (0.040) (-0.028 - 0.18)	-0.019, 0.19	0.091	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.10 (0.095) (3.91 - 4.23)	3.74 (0.095) (3.38 - 3.98)	0.36 (0.10) (0.20 - 0.79)	0.12, 0.61	0.011	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	508.96 (2.44) (506.18 - 511.92)	503.38 (2.44) (499.09 - 512.65)	5.58 (3.45) (-4.16 - 9.98)	-2.87, 14.02	0.157	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	47.15 (0.54) (46.17 - 48.81)	48.67 (0.54) (47.50 - 49.59)	-1.52 (0.75) (-3.27 - -0.78)	-3.36, 0.32	0.090	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.48 (0.17) (6.16 - 6.98)	7.08 (0.17) (6.63 - 7.37)	-0.60 (0.19) (-1.04 - -0.17)	-1.07, -0.14	0.019	4.79, 9.92 (6.05 - 10.50)
Protein	23.61 (0.42) (22.49 - 24.37)	23.92 (0.42) (23.56 - 24.61)	-0.31 (0.49) (-1.23 - 0.78)	-1.52, 0.89	0.547	22.30, 29.41 (20.58 - 29.28)
Total Fat	25.13 (0.44) (24.62 - 25.54)	23.65 (0.44) (22.92 - 25.20)	1.48 (0.63) (-0.15 - 2.28)	-0.061, 3.02	0.057	15.01, 28.51 (16.58 - 25.25)

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	25.58 (0.44) (24.19 - 26.37)	27.04 (0.44) (26.24 - 27.74)	-1.46 (0.60) (-3.49 - 0.082)	-2.93, 0.0044	0.050	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	19.49 (0.80) (18.47 - 20.73)	19.13 (0.80) (16.91 - 21.70)	0.36 (1.13) (-3.23 - 3.82)	-2.41, 3.12	0.763	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	32.88 (0.67) (31.85 - 34.31)	33.60 (0.67) (32.74 - 35.52)	-0.73 (0.95) (-3.28 - 0.90)	-3.04, 1.59	0.472	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	41.46 (0.62) (40.03 - 43.22)	41.87 (0.62) (40.16 - 43.29)	-0.40 (0.88) (-3.26 - 2.04)	-2.55, 1.74	0.661	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	0.93 (0.022) (0.88 - 0.98)	0.94 (0.022) (0.88 - 0.97)	-0.011 (0.029) (-0.059 - 0.10)	-0.083, 0.061	0.724	0.86, 1.11 (0.83 - 1.22)
Arginine	2.51 (0.088) (2.31 - 2.67)	2.59 (0.088) (2.41 - 2.71)	-0.075 (0.10) (-0.20 - 0.26)	-0.32, 0.17	0.482	2.38, 3.47 (2.30 - 3.55)

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.03 (0.054) (1.95 - 2.12)	2.07 (0.054) (1.92 - 2.18)	-0.037 (0.070) (-0.17 - 0.20)	-0.21, 0.13	0.610	1.94, 2.57 (1.79 - 2.72)
Cystine	0.36 (0.017) (0.33 - 0.38)	0.35 (0.017) (0.31 - 0.39)	0.0031 (0.018) (-0.026 - 0.066)	-0.041, 0.047	0.868	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	3.96 (0.13) (3.79 - 4.14)	4.10 (0.13) (3.66 - 4.40)	-0.14 (0.18) (-0.61 - 0.47)	-0.57, 0.30	0.473	3.74, 5.28 (3.39 - 5.45)
Glycine	0.96 (0.022) (0.92 - 1.00)	0.98 (0.022) (0.91 - 1.02)	-0.023 (0.031) (-0.087 - 0.088)	-0.10, 0.054	0.494	0.90, 1.14 (0.85 - 1.23)
Histidine	0.64 (0.021) (0.58 - 0.68)	0.64 (0.021) (0.61 - 0.66)	-0.00060 (0.025) (-0.058 - 0.064)	-0.062, 0.061	0.981	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.81 (0.023) (0.76 - 0.85)	0.82 (0.023) (0.77 - 0.83)	-0.0090 (0.030) (-0.067 - 0.074)	-0.082, 0.064	0.774	0.75, 0.96 (0.72 - 1.03)

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.34 (0.034) (1.28 - 1.42)	1.36 (0.034) (1.28 - 1.40)	-0.014 (0.046) (-0.071 - 0.14)	-0.13, 0.099	0.770	1.25, 1.62 (1.20 - 1.72)
Lysine	1.08 (0.027) (1.03 - 1.12)	1.11 (0.027) (1.06 - 1.17)	-0.031 (0.025) (-0.066 - 0.049)	-0.092, 0.031	0.269	1.01, 1.30 (0.99 - 1.44)
Methionine	0.36 (0.016) (0.33 - 0.41)	0.33 (0.016) (0.32 - 0.35)	0.030 (0.020) (-0.012 - 0.088)	-0.020, 0.080	0.188	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.20 (0.033) (1.13 - 1.25)	1.23 (0.033) (1.15 - 1.27)	-0.029 (0.039) (-0.10 - 0.098)	-0.12, 0.066	0.480	1.12, 1.58 (1.10 - 1.63)
Proline	0.86 (0.026) (0.78 - 0.89)	0.87 (0.026) (0.81 - 0.93)	-0.012 (0.026) (-0.080 - 0.077)	-0.074, 0.051	0.665	0.83, 1.08 (0.79 - 1.17)
Serine	0.94 (0.028) (0.93 - 0.96)	0.96 (0.028) (0.86 - 1.03)	-0.013 (0.033) (-0.11 - 0.098)	-0.094, 0.068	0.710	0.83, 1.21 (0.81 - 1.24)

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.78 (0.019) (0.73 - 0.82)	0.77 (0.019) (0.73 - 0.81)	0.0049 (0.023) (-0.028 - 0.086)	-0.052, 0.062	0.840	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.36 (0.0093) (0.34 - 0.38)	0.38 (0.0093) (0.37 - 0.40)	-0.019 (0.0085) (-0.038 - 0.010)	-0.040, 0.0015	0.063	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.71 (0.020) (0.68 - 0.74)	0.71 (0.020) (0.67 - 0.74)	-0.0059 (0.025) (-0.035 - 0.073)	-0.068, 0.056	0.822	0.67, 0.84 (0.63 - 0.91)
Valine	1.05 (0.029) (0.98 - 1.11)	1.07 (0.029) (1.00 - 1.10)	-0.013 (0.039) (-0.10 - 0.11)	-0.11, 0.082	0.751	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.71 (0.011) (0.69 - 0.74)	0.73 (0.011) (0.72 - 0.75)	-0.013 (0.012) (-0.033 - 0.013)	-0.042, 0.015	0.305	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	24.54 (0.086) (24.37 - 24.64)	24.39 (0.086) (24.07 - 24.59)	0.15 (0.12) (-0.12 - 0.56)	-0.15, 0.44	0.278	16.54, 30.55 (19.11 - 26.73)

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.49 (0.0056) (0.47 - 0.50)	0.48 (0.0056) (0.47 - 0.49)	0.0028 (0.0076) (-0.016 - 0.024)	-0.016, 0.021	0.726	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.79 (0.036) (2.72 - 2.93)	2.67 (0.036) (2.58 - 2.76)	0.12 (0.050) (0.033 - 0.24)	-0.0034, 0.24	0.054	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	14.44 (0.11) (14.05 - 14.68)	14.46 (0.11) (14.42 - 14.49)	-0.019 (0.15) (-0.40 - 0.20)	-0.39, 0.35	0.901	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.63 (0.18) (55.18 - 56.00)	55.87 (0.18) (55.61 - 56.29)	-0.25 (0.25) (-0.66 - 0.24)	-0.86, 0.37	0.366	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.16 (0.0017) (0.15 - 0.16)	0.15 (0.0017) (0.14 - 0.15)	0.0098 (0.0023) (0.0036 - 0.014)	0.0042, 0.015	0.005	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.31 (0.0070) (0.30 - 0.31)	0.30 (0.0070) (0.29 - 0.30)	0.012 (0.0099) (0.00039 - 0.024)	-0.013, 0.036	0.281	0.17, 0.38 (0.20 - 0.36)

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.14 (0.0033) (0.13 - 0.15)	0.14 (0.0033) (0.13 - 0.14)	-0.0010 (0.0046) (-0.015 - 0.015)	-0.012, 0.010	0.836	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.11 (0.0040) (0.10 - 0.12)	0.091 (0.0040) (0.081 - 0.095)	0.020 (0.0056) (0.0064 - 0.030)	0.0058, 0.033	0.013	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	5.65 (0.24) (5.02 - 6.58)	5.64 (0.24) (5.40 - 5.85)	0.015 (0.32) (-0.43 - 0.73)	-0.78, 0.80	0.965	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	67.74 (4.68) (60.53 - 81.81)	73.46 (4.68) (63.01 - 89.93)	-5.73 (4.30) (-14.73 - 3.89)	-16.25, 4.80	0.231	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.38 (0.0090) (0.36 - 0.41)	0.36 (0.0090) (0.34 - 0.37)	0.026 (0.0080) (-0.00067 - 0.046)	0.0063, 0.045	0.017	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	12.44 (0.51) (10.59 - 13.87)	9.72 (0.51) (8.61 - 11.03)	2.73 (0.72) (-0.44 - 5.26)	0.97, 4.48	0.008	9.07, 17.33 (9.07 - 17.14)

Table E-35. Statistical Summary of Site SCEK Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.64 (0.022) (0.59 - 0.72)	0.63 (0.022) (0.58 - 0.68)	0.0061 (0.018) (-0.040 - 0.054)	-0.039, 0.051	0.751	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.14 (0.031) (1.11 - 1.19)	1.02 (0.031) (0.88 - 1.08)	0.12 (0.043) (0.027 - 0.31)	0.015, 0.23	0.031	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.018 (0.0033) (0.0054 - 0.025)	0.015 (0.0033) (0.012 - 0.023)	0.0028 (0.0046) (-0.018 - 0.013)	-0.0085, 0.014	0.562	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	29.14 (0.82) (27.60 - 30.85)	30.08 (0.82) (28.22 - 31.74)	-0.94 (0.82) (-2.60 - 1.99)	-2.96, 1.08	0.297	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	159.04 (1.77) (154.81 - 162.27)	158.20 (1.77) (153.15 - 162.63)	0.84 (2.50) (-7.82 - 7.76)	-5.29, 6.97	0.749	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-36. Statistical Summary of Site SCEK Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.15 (0.0087) (0.15 - 0.16)	0.15 (0.0087) (0.14 - 0.15)	0.0062 (0.012) (-0.0031 - 0.012)	-0.024, 0.036	0.632	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.43 (0.029) (0.40 - 0.48)	0.43 (0.029) (0.39 - 0.46)	-0.0044 (0.041) (-0.026 - 0.015)	-0.10, 0.096	0.918	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.014) (0.21 - 0.25)	0.24 (0.014) (0.22 - 0.25)	-0.011 (0.020) (-0.020 - -0.0016)	-0.060, 0.038	0.596	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	1.06 (0.029) (1.03 - 1.10)	1.13 (0.029) (1.06 - 1.20)	-0.064 (0.027) (-0.14 - 0.012)	-0.13, 0.0010	0.052	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.14 (0.025) (1.06 - 1.23)	1.07 (0.025) (1.05 - 1.10)	0.069 (0.031) (-0.00076 - 0.18)	-0.0076, 0.15	0.069	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	3.84 (0.051) (3.76 - 3.98)	3.46 (0.051) (3.34 - 3.61)	0.38 (0.072) (0.20 - 0.63)	0.20, 0.56	0.001	3.42, 4.65 (3.18 - 4.68)
Calories (Kcal/100g)	500.56 (2.54) (499.03 - 501.60)	494.42 (2.54) (489.10 - 500.98)	6.14 (3.47) (-0.76 - 12.43)	-2.35, 14.63	0.127	457.61, 527.56 (466.09 - 509.91)
Carbohydrates	44.20 (0.48) (43.36 - 44.54)	46.39 (0.48) (45.65 - 47.07)	-2.18 (0.64) (-3.71 - -1.24)	-3.75, -0.62	0.014	40.26, 56.45 (43.28 - 54.90)
Moisture (% fw)	6.86 (0.21) (6.30 - 7.24)	7.47 (0.21) (7.11 - 7.79)	-0.61 (0.29) (-0.89 - -0.29)	-1.32, 0.099	0.079	4.79, 9.92 (6.05 - 10.50)
Protein	28.77 (0.24) (28.28 - 29.48)	28.48 (0.24) (28.09 - 28.77)	0.30 (0.29) (-0.49 - 0.74)	-0.42, 1.01	0.348	22.30, 29.41 (20.58 - 29.28)
Total Fat	23.19 (0.48) (22.75 - 23.37)	21.70 (0.48) (20.71 - 22.88)	1.49 (0.65) (0.36 - 2.63)	-0.097, 3.09	0.061	15.01, 28.51 (16.58 - 25.25)

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Acid Detergent Fiber	24.53 (0.37) (23.82 - 25.07)	25.53 (0.37) (24.51 - 26.91)	-1.00 (0.41) (-2.23 - -0.13)	-2.01, 0.0086	0.051	22.24, 31.96 (23.42 - 31.62)
Crude Fiber	17.12 (0.38) (16.76 - 17.54)	18.10 (0.38) (17.35 - 19.63)	-0.98 (0.45) (-2.09 - -0.10)	-2.07, 0.11	0.070	16.93, 22.68 (16.92 - 23.32)
Neutral Detergent Fiber	30.70 (0.41) (30.40 - 31.02)	32.12 (0.41) (30.49 - 33.05)	-1.42 (0.58) (-2.03 - 0.031)	-2.84, 0.0015	0.050	27.03, 42.49 (29.27 - 40.63)
Total Dietary Fiber	40.66 (0.62) (38.95 - 42.48)	40.47 (0.62) (39.15 - 42.09)	0.19 (0.72) (-1.42 - 1.70)	-1.57, 1.95	0.797	34.52, 52.58 (37.29 - 48.60)
Amino Acid (% dw)						
Alanine	1.05 (0.019) (1.04 - 1.09)	1.05 (0.019) (0.97 - 1.10)	0.0027 (0.026) (-0.061 - 0.069)	-0.061, 0.067	0.920	0.86, 1.11 (0.83 - 1.22)
Arginine	3.15 (0.074) (3.10 - 3.24)	3.25 (0.074) (2.94 - 3.49)	-0.10 (0.10) (-0.37 - 0.18)	-0.36, 0.15	0.355	2.38, 3.47 (2.30 - 3.55)

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Aspartic Acid	2.47 (0.047) (2.42 - 2.55)	2.45 (0.047) (2.26 - 2.62)	0.027 (0.067) (-0.20 - 0.19)	-0.14, 0.19	0.702	1.94, 2.57 (1.79 - 2.72)
Cystine	0.41 (0.015) (0.39 - 0.44)	0.40 (0.015) (0.36 - 0.45)	0.0059 (0.017) (-0.019 - 0.050)	-0.035, 0.046	0.734	0.31, 0.45 (0.29 - 0.47)
Glutamic Acid	4.95 (0.13) (4.85 - 5.11)	5.02 (0.13) (4.41 - 5.32)	-0.068 (0.19) (-0.48 - 0.57)	-0.53, 0.39	0.728	3.74, 5.28 (3.39 - 5.45)
Glycine	1.11 (0.022) (1.08 - 1.14)	1.11 (0.022) (1.03 - 1.19)	-0.0046 (0.031) (-0.086 - 0.050)	-0.081, 0.071	0.886	0.90, 1.14 (0.85 - 1.23)
Histidine	0.74 (0.018) (0.70 - 0.77)	0.76 (0.018) (0.71 - 0.82)	-0.013 (0.026) (-0.047 - 0.034)	-0.075, 0.050	0.635	0.59, 0.81 (0.57 - 0.84)
Isoleucine	0.92 (0.013) (0.89 - 0.94)	0.93 (0.013) (0.89 - 0.97)	-0.010 (0.017) (-0.058 - 0.022)	-0.051, 0.031	0.569	0.75, 0.96 (0.72 - 1.03)

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Leucine	1.55 (0.026) (1.52 - 1.58)	1.55 (0.026) (1.45 - 1.64)	0.00049 (0.037) (-0.090 - 0.10)	-0.091, 0.092	0.989	1.25, 1.62 (1.20 - 1.72)
Lysine	1.22 (0.026) (1.17 - 1.26)	1.24 (0.026) (1.19 - 1.33)	-0.016 (0.037) (-0.066 - 0.018)	-0.11, 0.075	0.680	1.01, 1.30 (0.99 - 1.44)
Methionine	0.37 (0.021) (0.33 - 0.40)	0.39 (0.021) (0.32 - 0.44)	-0.018 (0.029) (-0.068 - 0.051)	-0.090, 0.053	0.553	0.32, 0.38 (0.29 - 0.49)
Phenylalanine	1.48 (0.034) (1.47 - 1.52)	1.48 (0.034) (1.36 - 1.61)	0.00099 (0.048) (-0.13 - 0.11)	-0.12, 0.12	0.984	1.12, 1.58 (1.10 - 1.63)
Proline	1.05 (0.024) (1.02 - 1.08)	1.04 (0.024) (1.01 - 1.11)	0.012 (0.033) (-0.074 - 0.076)	-0.069, 0.094	0.724	0.83, 1.08 (0.79 - 1.17)
Serine	1.12 (0.031) (1.07 - 1.17)	1.11 (0.031) (0.97 - 1.17)	0.0077 (0.044) (-0.11 - 0.14)	-0.10, 0.12	0.867	0.83, 1.21 (0.81 - 1.24)

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Threonine	0.88 (0.016) (0.87 - 0.90)	0.86 (0.016) (0.80 - 0.91)	0.020 (0.022) (-0.042 - 0.075)	-0.034, 0.073	0.407	0.72, 0.89 (0.67 - 0.96)
Tryptophan	0.41 (0.013) (0.39 - 0.43)	0.43 (0.013) (0.38 - 0.47)	-0.013 (0.018) (-0.079 - 0.051)	-0.058, 0.032	0.502	0.34, 0.42 (0.31 - 0.46)
Tyrosine	0.82 (0.017) (0.81 - 0.85)	0.81 (0.017) (0.74 - 0.87)	0.015 (0.024) (-0.042 - 0.074)	-0.043, 0.074	0.546	0.67, 0.84 (0.63 - 0.91)
Valine	1.26 (0.030) (1.19 - 1.38)	1.23 (0.030) (1.16 - 1.29)	0.029 (0.042) (-0.072 - 0.15)	-0.074, 0.13	0.513	1.00, 1.28 (0.97 - 1.36)
Fatty Acid (% Total FA)						
14:0 Myristic	0.83 (0.010) (0.81 - 0.85)	0.84 (0.010) (0.81 - 0.87)	-0.013 (0.013) (-0.063 - 0.019)	-0.046, 0.020	0.358	0.16, 1.37 (0.45 - 1.04)
16:0 Palmitic	23.24 (0.14) (22.99 - 23.40)	23.01 (0.14) (22.86 - 23.25)	0.22 (0.16) (0.045 - 0.54)	-0.18, 0.62	0.219	16.54, 30.55 (19.11 - 26.73)

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.47 (0.0058) (0.45 - 0.48)	0.46 (0.0058) (0.45 - 0.47)	0.0043 (0.0072) (-0.017 - 0.019)	-0.013, 0.022	0.575	0.39, 0.70 (0.44 - 0.67)
18:0 Stearic	2.31 (0.025) (2.26 - 2.34)	2.46 (0.025) (2.40 - 2.52)	-0.15 (0.028) (-0.24 - -0.090)	-0.22, -0.083	0.001	1.98, 2.95 (1.98 - 2.97)
18:1 Oleic	16.14 (0.078) (16.04 - 16.22)	16.16 (0.078) (15.86 - 16.44)	-0.021 (0.11) (-0.22 - 0.31)	-0.29, 0.25	0.852	11.38, 20.64 (13.71 - 18.39)
18:2 Linoleic	55.63 (0.21) (55.38 - 55.91)	55.58 (0.21) (55.18 - 56.13)	0.050 (0.29) (-0.74 - 0.44)	-0.67, 0.77	0.868	47.49, 63.18 (49.78 - 59.61)
18:3 Linolenic	0.16 (0.0056) (0.14 - 0.18)	0.17 (0.0056) (0.16 - 0.17)	-0.0030 (0.0079) (-0.032 - 0.012)	-0.022, 0.016	0.714	0.060, 0.24 (0.10 - 0.29)
20:0 Arachidic	0.26 (0.0055) (0.24 - 0.28)	0.28 (0.0055) (0.28 - 0.29)	-0.018 (0.0078) (-0.049 - -0.0036)	-0.037, 0.00061	0.055	0.17, 0.38 (0.20 - 0.36)

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
22:0 Behenic	0.16 (0.0015) (0.15 - 0.16)	0.16 (0.0015) (0.16 - 0.16)	0.0019 (0.0020) (-0.0028 - 0.0060)	-0.0030, 0.0067	0.385	0.070, 0.21 (0.051 - 0.19)
Mineral						
Calcium (% dw)	0.16 (0.0016) (0.16 - 0.17)	0.14 (0.0016) (0.13 - 0.14)	0.024 (0.0023) (0.018 - 0.033)	0.019, 0.030	<0.001	0.058, 0.21 (0.081 - 0.18)
Copper (mg/kg dw)	10.33 (0.15) (9.96 - 10.53)	9.98 (0.15) (9.58 - 10.41)	0.35 (0.22) (-0.45 - 0.83)	-0.18, 0.88	0.156	2.97, 12.86 (4.46 - 11.62)
Iron (mg/kg dw)	69.52 (5.67) (59.77 - 92.17)	79.02 (5.67) (67.45 - 95.10)	-9.50 (7.06) (-14.73 - -2.93)	-26.76, 7.77	0.227	47.30, 97.12 (39.49 - 114.34)
Magnesium (% dw)	0.36 (0.0045) (0.35 - 0.37)	0.34 (0.0045) (0.33 - 0.34)	0.025 (0.0040) (0.015 - 0.032)	0.015, 0.035	<0.001	0.28, 0.47 (0.31 - 0.46)
Manganese (mg/kg dw)	11.38 (0.34) (10.73 - 12.83)	9.04 (0.34) (8.83 - 9.54)	2.34 (0.48) (1.35 - 4.00)	1.17, 3.51	0.002	9.07, 17.33 (9.07 - 17.14)

Table E-37. Statistical Summary of Site TXPL Cottonseed Nutrients for MON 88701 (Not Treated) vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (% dw)	0.58 (0.0099) (0.58 - 0.59)	0.57 (0.0099) (0.54 - 0.60)	0.0062 (0.011) (-0.020 - 0.032)	-0.020, 0.032	0.582	0.49, 0.87 (0.48 - 0.87)
Potassium (% dw)	1.03 (0.023) (0.99 - 1.09)	0.87 (0.023) (0.79 - 0.93)	0.16 (0.033) (0.061 - 0.30)	0.079, 0.24	0.002	0.92, 1.21 (0.90 - 1.26)
Sodium (% dw)	0.018 (0.010) (0.0054 - 0.024)	0.047 (0.010) (0.019 - 0.090)	-0.029 (0.014) (-0.085 - 0.0040)	-0.063, 0.0062	0.091	0, 0.066 (0.0054 - 0.077)
Zinc (mg/kg dw)	34.14 (0.45) (33.08 - 35.14)	34.96 (0.45) (33.70 - 35.89)	-0.82 (0.64) (-2.58 - 0.54)	-2.39, 0.76	0.250	27.27, 44.95 (25.07 - 48.49)
Vitamin (mg/kg dw)						
Vitamin E	110.33 (2.75) (103.52 - 114.05)	103.66 (2.75) (93.92 - 109.90)	6.67 (3.90) (-6.38 - 20.14)	-2.86, 16.20	0.137	41.91, 205.89 (84.07 - 162.76)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table E-38. Statistical Summary of Site TXPL Cottonseed Anti-nutrients for MON 88701 (Not Treated) vs. Conventional Control

Analytical Component (Units)[1]	MON 88701[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88701 minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Cyclopropenoid Fatty Acid (% Total FA)						
Dihydrosterculic Acid	0.16 (0.0047) (0.16 - 0.17)	0.16 (0.0047) (0.15 - 0.17)	-0.0020 (0.0066) (-0.0083 - 0.0048)	-0.018, 0.014	0.777	0.078, 0.25 (0.038 - 0.23)
Malvalic Acid	0.42 (0.020) (0.40 - 0.44)	0.47 (0.020) (0.44 - 0.49)	-0.046 (0.029) (-0.072 - -0.018)	-0.12, 0.025	0.161	0.23, 0.54 (0.11 - 0.59)
Sterculic Acid	0.23 (0.013) (0.21 - 0.25)	0.26 (0.013) (0.25 - 0.27)	-0.025 (0.018) (-0.043 - 0.0078)	-0.068, 0.019	0.216	0.17, 0.27 (0.061 - 0.34)
Gossypol (% dw)						
Free Gossypol	0.98 (0.024) (0.94 - 1.05)	0.93 (0.024) (0.91 - 0.95)	0.052 (0.033) (0.0089 - 0.14)	-0.030, 0.13	0.170	0.099, 1.57 (0.50 - 1.41)
Total Gossypol	1.09 (0.024) (1.03 - 1.16)	1.01 (0.024) (0.97 - 1.05)	0.081 (0.022) (0.053 - 0.11)	0.028, 0.13	0.009	0.064, 1.76 (0.56 - 1.61)

[1]dw = dry weight; FA = fatty acid.
[2] MON 88701 plants were not treated with dicamba or glufosinate.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control (Coker 130).
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

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Appendix F: Materials, Methods, and Individual Site Results for Seed Dormancy and Germination Assessment of MON 88701

F.1. Materials

Seed dormancy and germination characteristics were assessed on seed from MON 88701, the conventional control, and commercial reference varieties produced in 2010 field trials at the following sites: Crittenden County, Arkansas (ARPR); Caswell County, North Carolina (NCME); and Hale County, Texas (TXPL) in 2010 (Table VII-3). The field trial at each site was established in a randomized complete block design with four replications. The seed from MON 88701, the conventional control, and the commercial reference varieties were harvested from all four replicated plots at each of the three field sites.

Table F-1. Starting Seed of MON 88701, Conventional Control and Commercial Cotton Reference Varieties Used in Dormancy Assessment

Site[1]	Material	Material Type	Phenotype	Sample ID
ARPR	Coker 130	Control	Conventional	11268128
ARPR	MON 88701	Test	DGT Cotton[1]	11268129
ARPR	SG 125	Reference	Conventional	11266155
ARPR	DP 565	Reference	Conventional	11266764
ARPR	ST 474	Reference	Conventional	11266156
ARPR	DP 5415	Reference	Conventional	11266157
NCME	Coker 130	Control	Conventional	11268128
NCME	MON 88701	Test	DGT Cotton	11268129
NCME	DP 435	Reference	Conventional	11266762
NCME	Delta Opal	Reference	Conventional	11266158
NCME	SG 125	Reference	Conventional	11266155
NCME	FM 989	Reference	Conventional	10001810
TXPL	Coker 130	Control	Conventional	11268128
TXPL	MON 88701	Test	DGT Cotton	11268129
TXPL	Atlas	Reference	Conventional	11266765
TXPL	DP 435	Reference	Conventional	11266762
TXPL	SG 125	Reference	Conventional	11266155
TXPL	NM 1517-99	Reference	Conventional	11268233

[1]DGT Cotton = Dicamba and glufosinate-tolerant cotton.

F.2. Characterization of the Materials

For the MON 88701, the parental conventional control, and the commercial reference varieties starting seed lots, the presence or absence of the MON 88701 insert was confirmed by event-specific polymerase chain reaction analyses.

F.3. Germination Testing Facility and Experimental Methods

Seed dormancy and germination evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator was qualified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association (AOSA, 2010a; b; AOSA/SCST, 2010).

Seed lots of MON 88701, the conventional control, and four commercial reference varieties were produced from each of three field sites and tested under six different temperature regimes. Each temperature regime constituted a different experiment (*i.e.*, no comparisons were made between temperature regimes). Six germination chambers were maintained dark under one of the following temperature regimes: constant temperature of approximately 10, 20, or 30 °C, or alternating temperatures of approximately 10/20, 10/30, or 20/30 °C. The alternating temperature regimes were maintained at the lower temperature for 16 hours and the higher temperature for eight hours. The temperature inside each germination chamber was monitored and recorded every 15 minutes throughout the duration of the assessment. Prior to the study, the starting seed were treated uniformly with the commercial seed treatment fungicides mefenoxam and fludioxonil at labeled rates. Approximately 100 cottonseeds were placed on the germination towels using a vacuum planting system. Two additional pre-moistened germination towels were placed on top of the cottonseed. The set was rolled up and secured with a rubber band. All rolled germination towels were labeled and then placed into an appropriately labeled bucket. One germination towel was prepared for each of the six starting cottonseed entries from each individual site, all of which were placed into a single bucket. A bucket was prepared for each site and replicaton for a total of 12 buckets per temperature regime. Buckets were placed in the appropriate germination chambers.

A description of each germination characteristic evaluated and the timing of evaluations are presented in Table VII-1. The types of data collected depended on the temperature regime. Each rolled germination towel in the AOSA-recommended temperature regime (*i.e.*, 20/30°C) was evaluated periodically during the study for normal germinated, abnormal germinated, viable hard, dead, and viable firm-swollen seed as defined by AOSA guidelines (AOSA, 2010a; b; AOSA/SCST, 2010). AOSA only provides guidelines (AOSA, 2010a) for testing seed under optimal temperatures (20/30°C); however, additional temperature regimes were included to test a range of temperature conditions. Each rolled germination towel in the additional temperature regimes (*i.e.*, 10, 20, 30, 10/20, and 10/30°C) was evaluated periodically for germinated, viable hard, dead, and viable firm-swollen seed. Emergence and/or development of essential structures of seedlings that otherwise would be categorized as "normal germinated" under optimal

temperature conditions may not be so at non-optimal temperatures. Therefore, for the additional temperature regimes, no distinction was made between normal and abnormal germinated seed.

F.4. Statistical Analysis

An analysis of variance was conducted using SAS® Version 9.2 (SAS, 2008) according to a split-plot design with four replications. MON 88701 was compared to the conventional control for dormancy and germination characteristics of cottonseed produced within each site (*i.e.*, individual site analysis) and in a combined-site analysis in which the data were pooled across all three sites. The seed germination characteristics analyzed included percent germinated seed, percent viable hard seed, percent dead seed, and percent viable firm-swollen seed. The percent germinated seed were categorized as either normal germinated or abnormal germinated for the AOSA temperature regime. The level of statistical significance was predetermined to be 5% (α=0.05). MON 88701 was not statistically compared to the reference varieties, nor were comparisons made across temperature regimes. The minimum and maximum mean values were determined from the reference materials across all sites to provide a range of values (*i.e.*, reference range) representative of commercial cotton varieties. Results from the combined-site analysis are presented in Table VII-2.

F.5. Individual Site Seed Dormancy and Germination Analyses

In the individual site analyses, no statistically significant differences were detected between MON 88701 and the parental conventional control for any of the measured characteristics (*i.e.*, percent germinated, viable hard, dead, or viable firm-swollen seed) in any temperature regime for seed produced at the ARPR and NCME sites. Three statistically significant differences in total were detected between MON 88701 and the conventional control for cottonseed produced at the TXPL site (Table F-2). At TXPL, MON 88701 had fewer dead seed than the conventional control at 10/20°C (4.5% vs. 9.5%); MON 88701 had more germinated seed than the conventional control at 20/30°C (92.5% vs. 84.0%); and, MON 88701 had fewer abnormal germinated seed than the conventional control at 20/30°C (2.5% vs. 7.3%). Statistically significant differences between MON 88701 and the conventional control for germination characteristics in the individual site analyses were not consistently detected across temperature regimes or the individual sites. While some statistically significant differences were detected in the combined-site analysis, these statistical differences were within the range of values expected for the commercial reference varieties. Thus, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control. (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no").

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Table F-2. Comparison of MON 88701 to the Conventional Control for Dormancy and Germination Characteristics of Cottonseed Produced at Each of Three Sites

Temperature Regime	Germination Category	ARPR[1] Mean % (S.E.)[2]		NCME[1] Mean % (S.E.)[2]		TXPL[1] Mean % (S.E.)[2]	
		MON 88701	Control	MON 88701	Control[3]	MON 88701	Control
10 °C	Germinated	49.5 (13.5)	41.8 (11.2)	22.0 (7.2)	24.7 (4.2)	27.0 (7.6)	35.5 (5.5)
	Viable Hard	0.0 (0.0)	0.3 (0.3)	0.0 (0.0)	0.7 (0.7)	0.0 (0.0)	0.0 (0.0)
	Dead	16.0 (5.0)	15.0 (3.6)	32.0 (6.5)	26.7 (3.9)	20.5 (6.9)	25.8 (7.3)
	Viable Firm Swollen	34.5 (15.7)	43.0 (13.7)	46.0 (12.2)	48.0 (5.8)	52.5 (11.8)	38.8 (10.3)
20 °C	Germinated	96.0 (0.7)	96.8 (0.5)	94.5 (1.6)	97.3 (0.9)	96.5 (1.5)	92.3 (3.0)
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	4.0 (0.7)	3.3 (0.5)	5.5 (1.6)	2.7 (0.9)	3.5 (1.5)	7.8 (3.0)
	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)
30 °C	Germinated	99.0 (0.7)	97.0 (0.9)	95.8 (1.7)	93.7 (1.9)	95.2 (1.2)	92.3 (2.5)
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	1.0 (0.7)	3.0 (0.9)	4.3 (1.7)	6.3 (1.9)	4.8 (1.2)	7.8 (2.5)
	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)

Table F-2. Comparison of MON 88701 to the Conventional Control for Dormancy and Germination Characteristics of Cottonseed Produced at Each of Three Sites (continued)

Temperature Regime	Germination Category	ARPR [1] Mean % (S.E.)[2]		NCME [1] Mean % (S.E.)[2]		TXPL [1] Mean % (S.E.)[2]	
		MON 88701	Control	MON 88701	Control[3]	MON 88701	Control
10/20 °C	Germinated	98.0 (0.7)	95.3 (1.1)	90.3 (2.5)	90.3 (3.9)	95.0 (1.2)	90.0 (2.1)
	Viable Hard	0.0 (0.0)	0.3 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	2.0 (0.7)	4.3 (0.9)	6.5 (0.6)	6.0 (2.5)	4.5 (0.9)*	9.5 (2.1)
	Viable Firm Swollen	0.0 (0.0)	0.3 (0.3)	3.3 (2.9)	3.7 (1.5)	0.5 (0.5)	0.5 (0.3)
10/30 °C	Germinated	97.3 (0.8)	97.8 (0.9)	93.8 (2.7)	96.7 (0.9)	96.0 (1.4)	91.3 (3.5)
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	2.8 (0.8)	2.3 (0.9)	6.3 (2.7)	3.3 (0.9)	4.0 (1.4)	8.8 (3.5)
	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)
20/30 °C (AOSA)	Normal Germinated	92.0 (1.9)	94.5 (0.3)	84.2 (4.6)	84.7 (3.1)	92.5 (1.3)*	84.0 (7.2)
	Abnormal Germinated	3.8 (1.4)	2.5 (0.3)	8.0 (2.6)	9.0 (3.0)	2.5 (0.9)*	7.3 (2.2)
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	4.3 (1.5)	3.0 (0.6)	7.5 (2.0)	6.3 (0.9)	5.0 (0.7)	8.8 (5.5)
	Viable Firm Swollen	0.0 (0.0)	0.0 (0.0)	0.3 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Note: The experimental design for the germination test was a split-plot with four replications and statistical analysis consisted of an analysis of variance (ANOVA) model.

*Statistically significant differences detected ($\alpha = 0.05$) between MON 88701 and the conventional control (n = 11).

[1] In some instances, the total percentage of MON 88701 or the control did not equal 100% due to numerical rounding of the means.

[2] SE = Standard Error.

[3]Control – The NCME site only had 3 reps across all temperature regimes compared to 4 reps for other sites and treatments

References for Appendix F

AOSA. 2010. AOSA Rules for testing seeds Vol. 1: Principles and procedures. Association of Official Seed Analysts, Ithaca, New York.

AOSA. 2010. AOSA Rules for testing seeds Vol. 4: Seedling evaluation. Association of Official Seed Analysts, Ithaca, New York.

AOSA/SCST. 2010. Tetrazolium testing handbook. Association of Official Seed Analysts and the Society of Commercial Seed Technologists, Ithaca, New York.

Appendix G: Materials, Methods, Dicamba and Glufosinate Treated Results, and Individual Site Results from the Phenotypic, Agronomic, Plant Mapping, and Environmental Interaction Assessment of MON 88701 under Field Conditions

G.1. Materials

Data were collected from two different studies during 2010 to evaluate phenotypic, agronomic, and environmental interaction characteristics. In Study 1, MON 88701 not treated with dicamba or glufosinate herbicides was evaluated against the conventional control (Table G-1). In Study 2, MON 88701 not treated with dicamba or glufosinate herbicides and MON 88701 treated with dicamba- and glufosinate herbicides were both evaluated against the conventional control (Table G-2). Assessments were conducted on the agronomic system that includes MON 88701 treated with dicamba and glufosinate herbicides to support the assessment of MON 88701. In Study 1, a total of 11 cotton reference varieties were evaluated among the sites (Table G-1). Seven of the commercial varieties were conventional and four of the commercial varieties were tolerant to glyphosate herbicide. Three conventional reference varieties and one glyphosate-tolerant reference variety were grown at each location. In Study 2, a total of 8 commercial conventional reference varieties were evaluated among the sites. Four commercial conventional reference varieties were planted per site (Table G-2).

G.2. Characterization of the Materials

The presence or absence of the MON 88701 insert in the starting seed of MON 88701 and the conventional control were confirmed by event-specific polymerase chain reaction analyses.

G.3. Field Sites and Plot Design

Field sites in both studies (Table VII-3) are representative of commercial cotton growing areas and are distributed across a geographical area to include a variety of agronomic practices, soils and climatic factors. The researchers at each field site were familiar with the growth, production, and evaluation of cotton characteristics. The starting seed in each study were planted at each site in a randomized complete-block design with four replications.

Data from Study 1 were collected at fifteen field sites in the U.S. during 2010 (Table VII-3). Each plot (Table G-3) at the ARAU (Arkansas), ARPR (Arkansas), GACH (Georgia), LABU (Louisiana), and SCEK (South Carolina) consisted of 12 rows on 0.91 to 1.02 meter centers and rows were 9.1 meters long. In general, rows 2 and 3 were designated for the collection of following data: phenotypic, plant response to abiotic stress, disease damage, and arthropod damage. Rows 5 and 7 were designated for the collection of arthropod samples. Rows 9 and 10 were designated for the collection of damage data from thrips and heliothine spp. Rows 1, 4, 6, 8, 11, and 12 were used as buffer rows.

Each plot (Table G-3) at the ARTI (Arkansas), GAJE (Georgia), KSLA (Kansas), LACH (Louisiana), NCBD (North Carolina), NCME (North Carolina), NMGA (New Mexico), NMLC (New Mexico), TXPL (Texas), and TXPO (Texas) was 4 rows, row spacing of 0.76 to 1.02 meters and rows were 6.1 meters long. In general, rows 2 and 3 were designated for the collection of the following data: phenotypic, plant response to abiotic stress, disease damage, and arthropod damage. Rows 1 and 4 were used as buffer rows.

Data from Study 2 were collected at eleven field sites in the U.S. during 2010 (Section VII, Table VII-3). Each plot (Table G-4) was eight rows, row spacing was 0.76 to 1.02 meters and rows were 6 meters long. Rows 2 and 3 were designated for the collection of phenotypic, plant mapping and environmental interaction data.

G.4. Planting and Field Operations

Field and planting information for Study 1 and Study 2 are listed in Tables G-3 and G-4, respectively. Prior to planting, the Field Cooperator at each field site prepared the plot area with a proper seed bed according to local agronomic practices, including tillage, fertilization and pH adjustment, and pest management. During the growing season, all plots were assessed for agronomic conditions and pest populations, including pest arthropods, diseases, and weeds. Fertilizer, irrigation, agricultural chemicals and other management treatments were applied as necessary. All maintenance operations mentioned above were performed uniformly across all plots at a site.

Table G-1. Starting Seed for Study 1

Site[1]	Material Name	Phenotype[2]	Regulatory Lot Number
All	MON 88701	DGT	11268129
All	Coker 130	Control	11268128
ARTI	Phytogen Phy315RF	Glyphosate-Tolerant	11266967
ARTI	Delta Opal	Conventional	11266158
ARTI	DP435	Conventional	11266762
ARTI	ST474	Conventional	11266156
LABU	Bayer FM9058F	Glyphosate-Tolerant	11266968
LABU	DP565	Conventional	11266764
LABU	SG125	Conventional	11266155
LABU	ST474	Conventional	11266156
NCME	Nex Gen NG3410RF	Glyphosate-Tolerant	11266969
NCME	DP5415	Conventional	11266157
NCME	Delta Opal	Conventional	11266158
NCME	DP435	Conventional	11266762
GAJE	Bayer FM9058F	Glyphosate-Tolerant	11266968
GAJE	DP493	Conventional	11266763
GAJE	DP565	Conventional	11266764
GAJE	SG125	Conventional	11266155
NCBD	Phytogen Phy315RF	Glyphosate-Tolerant	11266967
NCBD	ST474	Conventional	11266156
NCBD	DP5415	Conventional	11266157
NCBD	Delta Opal	Conventional	11266158
TXPO	Bayer FM9058F	Glyphosate-Tolerant	11266968
TXPO	DP435	Conventional	11266762
TXPO	DP493	Conventional	11266763
TXPO	DP565	Conventional	11266764
TXPL	All tex patriotRF	Glyphosate-Tolerant	11266966
TXPL	SG125	Conventional	11266155
TXPL	ST474	Conventional	11266156
TXPL	DP5415	Conventional	11266157
ARPR	Nex Gen NG3410RF	Glyphosate-Tolerant	11266969
ARPR	ST474	Conventional	11266156
ARPR	DP493	Conventional	11266763
ARPR	SG125	Conventional	11266155
ARAU	Phytogen Phy315RF	Glyphosate-Tolerant	11266967
ARAU	Delta opal	Conventional	11266158
ARAU	DP435	Conventional	11266762
ARAU	ST474	Conventional	11266156
SCEK	Bayer FM9058F	Glyphosate-Tolerant	11266968
SCEK	SG125	Conventional	11266155
SCEK	ST474	Conventional	11266156
SCEK	DP5415	Conventional	11266157

Table G-1. Starting Seed for Study 1 (continued)

Site[1]	Material Name	Phenotype[2]	Regulatory Lot Number
GACH	Nex Gen NG3410RF	Glyphosate-Tolerant	11266969
GACH	Delta opal	Conventional	11266158
GACH	DP435	Conventional	11266762
GACH	DP493	Conventional	11266763
LACH	Phytogen Phy315RF	Glyphosate-Tolerant	11266967
LACH	DP5415	Conventional	11266157
LACH	ST474	Conventional	11266156
LACH	DP435	Conventional	11266762
KSLA	All tex patriotRF	Glyphosate-Tolerant	11266966
KSLA	Delta opal	Conventional	11266158
KSLA	DP435	Conventional	11266762
KSLA	DP493	Conventional	11266763
NMLC	Nex Gen NG3410RF	Glyphosate-Tolerant	11266969
NMLC	DP565	Conventional	11266764
NMLC	SG125	Conventional	11266155
NMLC	ST474	Conventional	11266156
NMGA	All tex patriotRF	Glyphosate-Tolerant	11266966
NMGA	DP5415	Conventional	11266157
NMGA	Delta opal	Conventional	11266158
NMGA	DP435	Conventional	11266762

[1] Sites - ARTI = Desha County, Arkansas; LABU = Rapides County, Louisiana; NCME = Caswell County, North Carolina; GAJE = Twiggs County, Georgia; NCBD = Perquimans County, North Carolina; TXPO = San Patricio County, Texas; TXPL = Hale County, Texas; ARPR = Crittenden County, Arkansas; ARAU = Jackson County, Arkansas; SCEK = Barnwell County, South Carolina; GACH = Tift County, Georgia; LACH = Rapides County, Louisiana; KSLA = Pawnee County, Kansas; NMLC = Dona Ana County, New Mexico; NMGA = Dona Ana County, New Mexico.

[2] Phenotype abbreviations: DGT = dicamba and glufosinate-tolerant.

Table G-2. Starting Seed for Study 2

Site[1]	Material Name[2]	Phenotype[3]	Regulatory Lot Number
All	Coker 130	Conventional	11268128
ARPR	SG125	Conventional	11266155
ARPR	DP 565	Conventional	11266764
ARPR	ST 474	Conventional	11266156
ARPR	DP 5415	Conventional	11266157
ARTI	SG125	Conventional	11266155
ARTI	DP 5415	Conventional	11266157
ARTI	DP 435	Conventional	11266762
ARTI	FM 989	Conventional	10001810
GACH	DP 565	Conventional	11266764
GACH	ST 474	Conventional	11266156
GACH	FM 989	Conventional	10001810
GACH	Delta Opal	Conventional	11266158
GAJE	SG125	Conventional	11266155
GAJE	ST 474	Conventional	11266156
GAJE	DP 5415	Conventional	11266157
GAJE	DP 435	Conventional	11266762
KSLA	DP 565	Conventional	11242914
KSLA	DP 5415	Conventional	11266157
KSLA	Atlas	Conventional	11266765
KSLA	NM 1517-99	Conventional	11268233
LACH	DP 565	Conventional	11266764
LACH	ST 474	Conventional	11266156
LACH	DP 5415	Conventional	11266157
LACH	FM 989	Conventional	10001810
NCBD	SG125	Conventional	11266155
NCBD	ST 474	Conventional	11266156
NCBD	DP 435	Conventional	11266762
NCBD	Delta Opal	Conventional	11266158
NCME	SG125	Conventional	11266155
NCME	DP 435	Conventional	11266762
NCME	FM 989	Conventional	10001810
NCME	Delta Opal	Conventional	11266158

Table G-2. Starting Seed for Study 2 (continued)

Site[1]	Material Name[2]	Phenotype[3]	Regulatory Lot Number
NMLC	DP 565	Conventional	11266764
NMLC	ST 474	Conventional	11266156
NMLC	Atlas	Conventional	11266765
NMLC	NM 1517-99	Conventional	11268233
SCEK	SG125	Conventional	11266155
SCEK	ST 474	Conventional	11266156
SCEK	Delta Opal	Conventional	11266158
SCEK	Atlas	Conventional	11266765
TXPL	SG125	Conventional	11266155
TXPL	DP 435	Conventional	11266762
TXPL	Atlas	Conventional	11266765
TXPL	NM 1517-99	Conventional	11268233
All	MON 88701 (U)	DGT	11268129
All	MON 88701 (S)	DGT	11268129

[1] ARPR= Crittenden County, Arkansas; ARTI = Desha County, Arkansas; GACH = Tift County, Georgia; GAJE = Twiggs County, Georgia; KSLA = Pawnee County, Kansas; LACH = Rapides County, Louisiana; NCBD = Perquimans County, North Carolina; NCME = Caswell County, North Carolina; NMLC = Dona Ana County, New Mexico; SCEK = Barnwell County, South Carolina; TXPL = Hale County, Texas.
[2] U = unsprayed, S = sprayed
[3]DGT = dicamba and glufosinate-tolerant.

Table G-3. Study 1 Field and Planting Information

Site	Planting Date[1]	Planting Rate (seeds/m)	Plot Size (m × m)[2]	Rows/plot	Soil Texture	% Organic Matter	2009 Cropping History
ARAU	5/25/2010	16	9.1 × 0.97	12	sandy loam	1.0	Soybean
ARPR	5/19/2010	16	9.1 × 0.91	12	silt loam	0.9	Corn
ARTI	5/24/2010	16	6.1 × 0.97	4	silt loam	2.2	Cotton
GACH	6/03/2010	16	9.1 × 0.91	12	loamy sand	1.0	Corn
GAJE	6/25/2010	16	6.1 × 0.91	4	loamy sand	0.8	Fallow
KSLA	6/02/2010	16	6.1 × 0.76	4	loam	2.1	Corn
LABU	5/20/2010	16	9.1 × 1.02	12	silt loam	0.6	Cotton
LACH	5/22/2010	16	6.1 × 1.02	4	very fine sandy	0.8	Soybean
NCBD	5/21/2010	16	6.1 × 0.97	4	loamy sand	1.6	Cotton
NCME	6/15/2010	16	6.1 × 0.76	4	loamy sand	0.9	Soybean
NMGA	5/20/2010	16	6.1 × 0.97	4	sandy loam	1.0	Cotton
NMLC	5/19/2010	16	6.1 × 0.97	4	loam	1.0	Cotton
SCEK	5/22/2010	16	9.1 × 1.02	12	loamy sand	1.5	Corn
TXPL	5/25/2010	16	6.1 × 1.02	4	clay loam	0.5	Corn
TXPO	5/21/2010	16	6.1 × 0.76	4	sandy clay loam	1.1	Corn

[1] Month-day-year.
[2] Length × width.

Table G-4. Study 2 Field and Planting Information

Site Code	Planting Date[1]	Planting Rate (seeds/m)	Plot Size (m × m)[2]	Rows/plot	Soil Texture	% Organic Matter	2009 Cropping History
ARPR	5/25/2010	16	6 × 0.91	8	Clay	1.3	Milo
ARTI	5/24/2010	16	6 × 0.97	8	Silt loam	1.6	Cotton
GACH	5/26/2010	16	6 × 0.91	8	Loamy sand	1.0	Soybean
GAJE	6/25/2010	16	6 × 0.91	8	Loamy sand	0.8	Fallow
KSLA	6/02/2010	16	6 × 0.76	8	Loam	3.1	Soybean
LACH	5/21/2010	16	6 x 1.02	8	Silt loam	0.6	Cotton
NCBD	5/27/2010	16	6 x 0.97	8	Loam	2.2	Cotton
NCME	6/11/2010	16	6 x 0.76	8	Loamy sand	1.0	Soybean
NMLC	5/17/2010	16	6 x 0.97	8	Sandy loam	1.0	Cotton
SCEK	5/19/2010	16	6 x 1.02	8	Loamy sand	1.3	Corn
TXPL	5/25/2010	16	6 x 1.02	8	Clay loam	0.5	Corn

[1] Month-day-year.
[2] Length × width.

G.5. Phenotypic Observations

In both Study 1 and Study 2, the description of the characteristics measured and the designated developmental stages when observations occurred are listed in Table VII-1.

G.6. Environmental Observations

In both Study 1 and Study 2, environmental interactions (*i.e.*, interactions between crop plants and their receiving environment) were used to characterize MON 88701 by evaluating plant response to abiotic stress, disease damage, and arthropod-related damage using qualitative methods described in section G.7. In addition, pest damage and pest- and beneficial-arthropod abundance were evaluated in Study 1 using the quantitative methods described in the following sections (G.7 and G.8).

G.7. Plant Response to Abiotic Stress, Disease Damage, and Arthropod-Related Damage

In Study 1 and Study 2, plots containing MON 88701 not treated with dicamba and glufosinate herbicides and the conventional control were evaluated qualitatively at all sites for differences in plant response to abiotic stress, disease damage, and arthropod-related damage. Three abiotic stressors, three diseases, and three arthropod pests were evaluated four times during the growing season at the following intervals:

Observation 1: approximately 30 days after planting (DAP)
Observation 2: approximately 60 DAP
Observation 3: approximately 90 DAP
Observation 4: approximately 120 DAP

Method used for selecting stressors at each field site:

1. Prior to each data collection, cotton was surveyed in proximity to the study area or the border rows of the study for abiotic stressors (*e.g.*, drought), diseases (*e.g.*, Alternaria black spot), and arthropod damage (*e.g.*, thrips).
2. Cooperators chose **three** abiotic stressors, **three** diseases, and **three** arthropod species that are actively causing damage for subsequent evaluation in the study plots. Cooperators were requested to select additional stressors if present.
3. If fewer than three abiotic stressors, diseases, or arthropod species were present, the cooperator chose additional abiotic stressors, diseases, and arthropod species that are known to commonly occur in that geographical region and cause damage at the study site at that time.
4. All plots at a site were rated for the same abiotic stressors, diseases, and arthropod pests at a given observation, even if that selected stressor was not present in some or all of the plots.
5. If a selected stressor was not present, the cooperator recorded the rating as "0" (= none).

As indicated above, the researcher at each field 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 cotton during the given observation period. Therefore, abiotic stressors, diseases, and arthropod pests assessed often varied between observations at a site and between sites. Qualitative plant response to abiotic stress and disease damage and arthropod-related damage observations were collected from each plot using a continuous 0 – 9 scale of increasing severity (in Study 2, qualitative abiotic and biotic stressor were not evaluated on plots with MON 88701 treated with dicamba and glufosinate herbicides). 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)

In Study 1, a quantitative assessment for differences in thrips and heliothine damage on MON 88701 and the conventional control plants was conducted at ARAU, ARPR, GACH, LABU, and SCEK.

Thrips damage was assessed quantitatively from rows 9 and 10 of each plot from 10 randomly selected plants using the arthropod-specific 0 – 5 rating scales of increasing severity listed below. Damage was rated at approximately 14, 21, and 28 DAP.

Rating	Severity of plant damage
0	No Thrips or damage visible
1	Few thrips present; no brownish tinge along the edges of leaves and silvering on the underside of leaves
2	Numerous thrips present; newest leaves show only a slight brownish tinge along the edges of leaves and some silvering on the underside of some leaves
3	Numerous thrips present; newest leaves show considerable browning along the edges of leaves and some silvering on the underside of most leaves
4	Numerous thrips present; extensive silvering of leaves with some curling of leaves
5	Numerous thrips present; extensive silvering of leaves, leaves often curl upwards and the plant is generally ragged in appearance

Heliothine damage was assessed from rows 9 and 10 of each plot. Visual observations were conducted at 45, 60, 75 and 90 DAP to record total number of fruiting bodies (flower buds, flowers and bolls), number of damaged fruiting bodies and number live larvae on the top 7 nodes from 10 randomly selected plants.

G.8. Arthropod Abundance

Pest and beneficial arthropods were collected at the ARAU, ARPR, GACH, LABU, and SCEK sites four times during the growing season at the following intervals:

Collection 1: 30 DAP
Collection 2: 60 DAP
Collection 3: 90 DAP
Collection 4: 120 DAP

Arthropods were collected using a vertical beat sheet sampling method (Drees and Rice, 1985). The beat sheet was approximately 0.91 × 0.91 m, constructed of a stiff material and had a collection trough at the bottom. The sheet was placed in a designated row and the collecting trough was positioned near the base of the plants. Plants were shaken vigorously along the length of the beat sheet to dislodge arthropods from the plants. This sample constituted a subsample. Two subsamples were collected form both rows 5 and 7 for a total of 4 subsamples per plot. The subsamples collected within the same row were at least 1.5 m apart. The four sub-samples were combined into one pre-labeled container and placed on freezer ice packs and sent to a laboratory to be enumerated.

A maximum of the five pest and five beneficial arthropods were enumerated for each collection. For each individual collection (*e.g.*, Collection 1, ARPR site), four randomly selected samples were examined to determine presence and relative abundance of up to five pest- and beneficial-arthropods to be enumerated for that particular collection and site. Thus, the suite of pest- and beneficial-arthropods assessed often varied between collections from a site and between sites due to differences in temporal activity and geographical distribution of arthropod taxa.

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. Study personnel assessed that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues during the trials that would impact the trial objectives were noted. Data were then subjected to categorical or statistical analysis as indicated G.10 (categorical analysis) and G.11 (statistical analysis).

G.10. Environmental Interactions Evaluation Criteria for Qualitative Data

The following data were categorical and not statistically analyzed: plant vigor at 14 DAP, plant vigor at 30 DAP, plant response to abiotic stress, disease damage and arthropod damage. MON 88701 and the conventional control were considered different in plant response rating if the range of vigor or stressor values did not overlap between the

MON 88701 and the conventional control across all four replications. Any observed differences between the MON 88701 and the conventional control were assessed for biological significance in the context of the range of the commercial reference materials, and for consistency in other observation times and sites. Differences that are not consistently observed at other times and sites are considered not biologically meaningful in terms of plant pest potential or an adverse environmental impact.

G.11. Statistical Analysis

Analysis of variance was conducted on the quantitative data according to a randomized complete block design using SAS® Version 9.2 (SAS, 2008). The level of significance was predetermined to be α=0.05. In separate analysis, MON 88701 not treated with dicamba or glufosinate herbicides from Study 1, MON 88701 not treated with dicamba or glufosinate herbicides from Study 2, and MON 88701 treated with dicamba and glufosinate herbicides were each compared to the conventional control within each site (individual site analysis) and in a combined-site analysis. In both Study 1 and Study 2, no statistical comparisons were made between MON 88701 and the reference varieties. The reference range for each characteristic analyzed across sites was determined from the minimum and maximum mean values from the reference cotton varieties planted among the sites, within a study.

MON 88701 not treated with dicamba or glufosinate herbicides from Study 1 was statistically compared to the conventional control within each site (individual site analysis) and in a combined-site analysis, in which the following data were pooled across sites: stand count at 14 DAP, stand count at 30 DAP, final stand count at harvest, plant height at 30 DAP, plant height at harvest, nodes above white flower (3 observations), seed cotton yield, seed index, total seed per boll, total mature seed per boll, total immature seed per boll, boll weight, fiber micronaire, fiber elongation, fiber strength, fiber uniformity, fiber length, thrips damage and percent heliothine damaged fruiting bodies and number of live heliothine larvae. Pest and beneficial arthropod abundance data were statistically analyzed only within individual observations/ collections and sites due to the variation in temporal activity and geographical distribution of the taxa. The reference range for pest- and beneficial-arthropod abundance and damage of each arthropod evaluated from a given collection/observation and site was determined from the minimum and maximum mean abundance or damage values collected from the reference varieties at the site. Data excluded from Study 1 (Table G-5) and the reasons for their exclusion are listed in the study file. Exclusion of these data did not adversely affect the quality of the study.

MON 88701 not treated with dicamba or glufosinate herbicide and MON 88701 treated with dicamba and glufosinate herbicides from Study 2 were each compared to the conventional control within each site (individual site analysis) and in a combined-site analysis, in which the following data were pooled across sites: stand count at 14 DAP, stand count at 30 DAP, final stand count, plant height at 30 DAP, plant height at harvest,

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nodes above white flower (3 observations), seed cotton yield, number of mainstem nodes per plant, number of nodes to first fruiting branch, total number of bolls per plant, total number of first-position bolls per plant, total number of vegetative bolls per plant, percent retention of first-position bolls, percent first-position bolls of total bolls per plant, seed index, total seed per boll, total mature seed per boll, total immature seed per boll, weight per boll, fiber micronaire, fiber elongation, fiber strength, fiber uniformity, and fiber length. Data excluded from Study 2 (Table G-6) and the reasons for their exclusion are listed in the study file. Exclusion of these data did not adversely affect the quality of the study.

G.12. Phenotypic Results from 2010 - Results and Discussion

The individual site data will be reported in three separate comparisons: Study 1 - MON 88701 not treated with dicamba or glufosinate herbicides vs. the conventional control; Study 2 - MON 88701 not treated with dicamba or glufosinate herbicides vs. the conventional control; and, Study 2 - MON 88701 treated with dicamba and glufosinate herbicides vs. the conventional control. The agronomic system that includes MON 88701 treated with dicamba and glufosinate herbicides was assessed to support the assessment of MON 88701.

G.12.1. Individual Phenotypic Characteristics - Results and Discussion for Study 1 - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides

In the individual site analysis of MON 88701 data, a total of 59 statistically significant differences were detected out of a total of 285 comparisons made between MON 88701 and the conventional control (Tables G-7, G-8 and G-9). At LACH (Table G-7), MON 88701 had a lower stand count (plants per plot) than the conventional control at 14 DAP (100.8 vs. 127.8), 30 DAP (101.8 vs. 125.0) and at harvest (63.3 vs. 73.5). At GAJE, the final stand count was higher for MON 88701 than the control (167.5 vs. 156.3 plants/plot). Plants of MON 88701 were shorter (cm) than the conventional control at 30 DAP at LACH (12.4 vs. 14.5), NCBD (20.0 vs. 24.5) and NMLC (6.7 vs. 8.3). Plants of MON 88701 were shorter (cm) than the control at harvest at GAJE (87.1 vs. 98.6), KSLA (129.9 vs. 142.0), LACH (120.5 vs. 140.5), NCBD (104.8 vs. 111.0), and TXPL (44.9 vs. 48.4). Plants of MON 88701 had more nodes above white flower at observation 1 at KSLA (5.1 vs. 4.4), NMLC (8.4 vs. 7.4) and TXPL (5.0 vs. 4.5). Plants of MON 88701 had more nodes above white flower at observation 2 at ARPR (7.1 vs. 6.6) and KSLA (4.2 vs. 3.2). Plants of MON 88701 had more nodes above white flower at observation 3 at KSLA (4.6 vs. 3.5), SCEK (5.2 vs. 4.6) and TXPO (6.4 vs. 5.7). Plants of MON 88701 a higher seedcotton yield (Kg/ha) than the conventional control at ARAU (2912.9 vs. 2166.8) and at GAJE (2320.4 vs. 1843.1). Plants of MON 88701 (Table G-8) had a lower seed index (grams of 100 fuzzy seed) than the conventional control ARPR (9.4 vs. 10.3), ARTI (10.1 vs. 10.9), GAJE (8.3 vs. 9.8), KSLA (11.8 vs. 12.5), LABU (9.4 vs. 10.5), LACH (8.8 vs. 9.8), NCBD (9.0 vs. 9.8), NCME (9.7 vs. 10.6), and NMGA (10.6 vs. 11.4). Plants of MON 88701 had more total seed per boll than the conventional control at ARAU (31.6 vs. 29.6), ARPR (29.4 vs. 27.3), KSLA (31.2 vs. 28.1), NCME (31.7 vs. 27.5), NMGA (34.4 vs. 30.3) and NMLC (31.0 vs. 25.9). Plants of MON 88701 had more mature seed per boll than the control at ARPR (27.3 vs. 21.7), ARTI (20.2 vs. 17.6),

KSLA (29.9 vs. 26.6), NCME (28.4 vs. 22.1), NMGA (32.9 vs. 28.8), and NMLC (26.5 vs. 21.6). Plants of MON 88701 had fewer immature seed per boll than the conventional control at ARPR (2.1 vs. 5.7), ARTI (5.7 vs. 9.0), and SCEK (6.5 vs. 11.1). Plants of MON 88701 (Table G-9) had lower boll weight (grams per boll) than the conventional control at ARTI (4.1 vs. 4.8) and GACH (4.2 vs. 4.5) and a higher boll weight at NMLC (5.1 vs. 4.6). Plants of MON 88701 had higher fiber micronaire (mic units) than the conventional control at NMGA (5.1 vs. 4.9) and TXPL (4.9 vs. 4.5). Plants of MON 88701 had greater fiber elongation (%) than the conventional control at ARAU (6.8 vs. 6.2) and lower fiber elongation at NCBD (5.7 vs. 6.6). Plants of MON 88701 had greater fiber strength (g/tex) than the conventional control at KSLA (31.5 vs. 30.3), NCBD (32.6 vs. 30.9), NMLC (31.2 vs. 30.0), and TXPL (34.1 vs. 32.2). Plants of MON 88701 had greater fiber uniformity than the conventional control at TXPL (85.4 vs. 84.1%) and shorter cotton fiber at the NCME (2.8 vs. 2.9 cm).

The statistical differences detected in the individual site analyses for stand count at 14 DAP, stand count at 30 DAP, final stand count at harvest, nodes above white flower at observation 1, seedcotton yield, immature seed per boll, boll weight, micronaire, fiber elongation, fiber uniformity, and fiber length were not detected in the combined-site analysis. Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no"). While statistical differences were detected in the combined-site analysis for plant height at 30 DAP at three sites, plant height at harvest at five sites, nodes above white flower observation 2 at two sites, and nodes above white flower observation 3 at three sites, seed index at nine sites, total seed per boll at six sites, and mature seed per boll at six sites and fiber strength at four sites, the assessed phenotypic value of MON 88701 for these phenotypic characteristics were within their respective reference range. Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no").

G.12.2. Individual Phenotypic Characteristics - Results and Discussion for Study 2 - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides

In the individual site analysis for MON 88701 data, 43 statistically significant differences were detected out of 209 comparisons between MON 88701 and the conventional control (Table G-10, G-11 and G-12). At 30 DAP, plants of MON 88701 were shorter (cm) than the conventional control at KSLA (15.7 vs. 18.3), LACH (13.5 vs. 15.9), and NCME (17.2 vs. 19.7 cm). At harvest, plants of MON 88701 were shorter (cm) than the conventional control at GAJE (77.4 vs. 92.6), KSLA (113.5 vs. 127.0 cm), LACH (154.4 vs. 180.0), and NCME (71.4 vs. 85.0). Plants of MON 88701 had more nodes above white flower than the conventional control at observation 1 at GACH (5.9 vs. 5.3), at

observation 2 at NCBD (4.8 vs. 3.9) and TXPL (5.5 vs. 5.1) and at observation 3 at GACH (4.1 vs. 3.6) and KSLA (3.7 vs. 2.5). Plants of MON 88701 had higher seedcotton yield than the conventional control at KSLA (4,487.0 vs. 3,726.5 kg/ha) and NMLC (1,938.4 vs. 1,479.3 kg/ha). Plants of MON 88701 had a lower seed index (gram of 100 seed) than the conventional control at ARPR (8.8 vs. 10.0), ARTI (9.9 vs. 12.0), GAJE (8.5 vs. 10.4), KSLA (11.7 vs. 12.8), LACH (9.4 vs. 10.3), NCBD (8.8 vs. 10.5), NCME (8.8 vs. 10.2), SCEK (8.5 vs. 9.8), and TXPL (9.9 vs. 11.0). Plants of MON 88701 had more total seed per boll than the conventional control at ARPR (26.8 vs. 23.9), KSLA (28.4 vs. 24.9), and NMLC (33.8 vs. 30.7). Plants of MON 88701 had more mature seed per boll than the conventional control at ARPR (24.2 vs. 15.9), ARTI (23.7 vs. 18.0), GAJE (15.0 vs. 11.6), and KSLA (25.9 vs. 22.7). Plants of MON 88701 had fewer immature seed per boll than the conventional control at ARPR (2.6 vs. 8.0) and ARTI (4.6 vs. 8.9). Plants of MON 88701 had greater weight per boll than the conventional control at KSLA (5.8 vs. 5.3 g/boll) and NMLC (5.7 vs. 5.1 g/boll). Plants of MON 88701 had higher fiber micronaire than the conventional control at NMLC (4.9 vs. 4.7 mic units), lower fiber elongation at ARPR (5.0 vs. 5.7 %), higher fiber strength (g/tex) at KSLA (30.4 vs. 29.4), NMLC (30.1 vs. 27.3), and TXPL (32.0 vs. 31.0). Plants of MON 88701 had higher fiber uniformity (%) than the conventional control at KSLA (84.2 vs. 83.3) and NMLC (83.4 vs. 81.1 %) and shorter fiber length (cm) at ARPR (2.8 vs. 2.9) and NCBD (2.8 vs. 2.9).

The statistical differences detected in the individual site analysis for nodes above white flower (observations 1 and 3), seedcotton yield, immature seed per boll, weight per boll, fiber micronaire, fiber elongation, fiber uniformity, and fiber length were not detected in the combined-site analysis. Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no"). Although statistical differences were detected for plant height at 30 DAP at three sites, plant height at harvest at four sites, nodes above white flower observation 2 at two sites, seed index at nine sites, total seed per boll at three sites, and mature seed per boll at four sites and fiber strength at three sites, the assessed values of MON 88701 for the combined-site analysis were within their respective reference range. Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no").

G.12.3. Combined-site and Individual Site Phenotypic Characteristics - Results and Discussion for Study 2 - MON 88701 Treated with Dicamba and Glufosinate Herbicides

To support the assessment of MON 88701, these assessments were also conducted on the agronomic system that includes MON 88701 treated with dicamba and glufosinate

herbicides. In the combined-site analysis (Table G-13), no statistically significant differences were detected between MON 88701 treated with dicamba and glufosinate herbicides and the conventional control for stand count at 14 DAP, stand count at 30 DAP, stand count at harvest, number of nodes above white flower observation 3, seedcotton yield, immature seed per boll, weight per boll, fiber micronaire, fiber elongation, fiber uniformity and fiber length. Therefore, the lack of differences in the above characteristics supports a conclusion that MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no"). The following statistically significant differences were detected in the combined-site analysis. Plants of MON 88701 were shorter (cm) than the conventional control at 30 DAP (18.1 vs. 19.2) and at harvest (98.4 vs. 105.0). Plants of MON 88701 had a higher number of nodes above white flower at observation 1 (6.7 vs. 6.4) and at observation 2 (5.6 vs. 5.2), lower seed index (9.5 vs. 10.7 g per 100 fuzzy seed), more seed per boll (28.5 vs. 27.0), more mature seed per boll (22.8 vs. 20.1), and, increased fiber strength (31.2 vs. 30.2 g/tex). However, the mean values for the above characteristics were within the reference range. Thus, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control.

In the individual site analysis for MON 88701 plant growth and development data (Table G-14 – G-16), a total of 41 statistically significant differences were detected out of a total of 209 comparisons made between treated MON 88701 and the conventional control. Plants of MON 88701 were shorter than the conventional control at 30 DAP at GAJE (23.2 vs. 26.0 cm). Plants of MON 88701 were shorter (cm) than the conventional control at harvest at GAJE (81.7 vs. 92.6), KSLA (113.6 vs. 127.0), LACH (164.8 vs.180.0) and NCME (75.7 vs. 85.0). Plants of MON 88701 had more nodes above white flower at observation 1 than the conventional control at LACH (8.6 vs. 7.6) and TXPL (6.2 vs. 5.7). Plants of MON 88701 had more nodes above white flower at observation 2 than the conventional control at GAJE (6.1 vs. 5.7), NCBD (5.7 vs. 3.9) and TXPL (5.7 vs. 5.1). Plants of MON 88701 had more nodes above white flower at observation 3 than the conventional control at ARTI (4.9 vs. 4.7), KSLA (3.4 vs. 2.5), and NCBD (3.8 vs. 3.0). Plants of MON 88701 had lower seedcotton yield than the conventional control at GACH (4,107.9 vs. 4,471.5 kg/ha) and higher at NMLC (2,048.0 vs. 1,479.3 kg/ha). Plants of MON 88701 had a lower seed index (g/100 fuzzy seed) than the conventional control at ARPR (9.1 vs. 10.0), ARTI (9.6 vs. 12.0), GACH (9.0 vs. 9.7), GAJE (8.1 vs. 10.4), LACH (9.5 vs. 10.3), NCBD (9.2 vs. 10.5), NCME (8.9 vs. 10.2), SCEK (8.5 vs. 9.8), and TXPL (9.9 vs. 11.0). Plants of MON 88701 had more total seed per boll than the conventional control at the ARPR (26.1 vs. 23.9) and GACH (29.7 vs. 27.4). Plants of MON 88701 had more mature seed per boll than the conventional control at ARPR (23.2 vs. 15.9), ARTI (22.5 vs. 18.0) and GACH (27.2 vs. 23.4). Plants of MON 88701 had fewer immature seed per boll than the conventional control at ARPR (2.9 vs. 8.0) and ARTI (5.3 vs. 8.9). Plants of MON 88701 had higher fiber micronaire than the control at NMLC (4.9 vs. 4.7 mic units) and higher fiber strength (g/tex) at GACH (31.0 vs. 29.5), KSLA (30.7 vs. 29.4), LACH (31.3 vs. 29.9), NMLC (29.3 vs. 27.3), SCEK (30.7 vs. 30.0) and the TXPL (33.0 vs. 31.0). Plants of MON 88701 had higher fiber uniformity

(%) than the conventional control at KSLA (84.3 vs. 83.3) and lower at SCEK (82.5 vs. 83.9). Plants of MON 88701 had shorter fiber length (cm) at SCEK (2.7 vs. 2.8).

The statistical differences detected in the individual site analysis for nodes above white flower observations 3 at three locations, seedcotton yield at two locations, immature seed per boll at two locations, fiber micronaire at one location, fiber uniformity at two locations and fiber length at one location were not detected in the combined-site analysis (Table G-13). Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control. (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no"). Although statistical differences were detected for plant height at 30 DAP at one site, plant height at harvest at four sites, nodes above white flower observation 1 at two sites, nodes above white flower observation 2 at three sites, seed index at nine sites, total seed per boll at two sites, and mature seed per boll at three sites and fiber strength index at 6 sites, the assessed values of MON 88701 for the combined-site analysis were within their respective reference range. Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no").

G.13. Plant Mapping Results and Discussion

G.13.1. Individual Site Plant Mapping Results for MON 88701 Not Treated with Dicamba or Glufosinate Herbicides - Results and Discussion.

In the individual site analysis, 14 statistically significant differences were detected out of 77 comparisons between MON 88701 and the control (Table G-17). Plants of MON 88701 had more mainstem nodes compared to the conventional control at ARTI (20.9 vs. 19.8) and a fewer number of nodes to the first fruiting branch at GACH (6.5 vs. 7.9) and GAJE (5.0 vs. 5.8). Plants of MON 88701 had fewer total bolls per plant compared to the conventional control at KSLA (19.1 vs. 20.6) and more total bolls at NCME (6.8 vs. 4.2) and NMLC (6.7 vs. 4.5). Plants of MON 88701 had more first-position bolls per plant compared to the conventional control at NCME (3.5 vs. 2.5), NMLC (4.4 vs. 3.0), and TXPL (7.8 vs. 7.1) and more vegetative bolls per plant at NCME (1.0 vs. 0.1). Plants of MON 88701 retained a higher percentage (%) of first-position bolls per plant compared to the conventional control at the NCME (35.6 vs. 23.9) and NMLC (30.1 vs. 21.3) and a higher percent of first-position bolls per plant relative to total bolls per plant at GAJE (67.4 vs. 61.8) and SCEK (73.2 vs. 65.7).

The statistical differences (Table G-17) detected between MON 88701 and the control in the individual site analysis for total mainstem nodes per plant at one location, the number of nodes per plant to the first fruiting branch at two sites, total bolls per plant at three sites, total vegetative bolls per plant at one site, percent first-position boll retention per plant at two sites and percent first-position bolls per plant relative to total bolls per plant

at two sites were not detected in the combined-site analysis (Table VII-6). Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no"). Although statistical differences were detected between the MON 88701 and the conventional control for total first-position bolls at three sites (Table G-17), the assessed values of MON 88701 for the combined-site analysis were within their respective reference range. Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no").

G.13.2. Combined-site and Individual Site Plant Mapping Results for MON 88701 Treated with Dicamba and Glufosinate Herbicides - Results and Discussion.

To support the assessment of MON 88701, plant mapping assessments were also conducted on the agronomic system that includes MON 88701 treated with dicamba and glufosinate herbicides. In the combined-site analysis (Table G-18) of plant mapping parameters, no statistically significant differences were detected between MON 88701 and the conventional control for total mainstem nodes per plant, the number of nodes per plant to the first fruiting branch, total bolls per plant, total vegetative bolls per plant, percent first-position boll retention per plant and percent first-position bolls per plant relative to total bolls per plant. Therefore, the lack of differences in the above characteristics supports a conclusion that MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no"). Plants of MON 88701 had a significantly higher number of first-position bolls per plant compared to the conventional control (5.2 vs. 4.6). However, the mean values of MON 88701 were within the reference range. Thus, MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control. (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no").

In the individual site analysis, 15 statistically significant differences were detected out of 77 comparisons between MON 88701 treated with dicamba and glufosinate herbicides compared to the conventional (Table G-19). Plants of MON 88701 had more mainstem nodes per plant compared to the conventional control at NCBD (16.5 vs. 14.8) and a fewer number of nodes to the first fruiting branch at GACH (6.5 vs. 7.9) and GAJE (5.1 vs. 5.8). Plants of MON 88701 had a higher number of total bolls per plant compared to the conventional control at NCME (7.1 vs. 4.2), NMLC (7.7 vs. 4.5) and TXPL (14.3 vs. 11.3) and a higher number of first-position bolls per plant at NCBD (6.5 vs. 5.5), NCME (3.4 vs. 2.5), NMLC (4.7 vs. 3.0) and TXPL (8.1 vs. 7.1). Plants of MON 88701 had

more vegetative bolls per plant compared to the convention control at NCME (1.1 vs. 0.1) and less at SCEK (0.3 vs. 0.7). Plants of MON 88701 retained a higher percent of first-position bolls compared to the control at NCME (33.7 vs. 23.9) and NMLC (32.2 vs. 21.3) and had a higher percentage of first-position bolls per plant relative to total bolls per plant at the GACH (45.4 vs. 36.6).

The statistical differences detected in the individual site analysis (Table G-19) for total mainstem nodes per plant, the number of nodes per plant to the first fruiting branch, total bolls per plant, total vegetative bolls per plant, percent first-position boll retention per plant and percent first-position bolls per plant relative to total bolls per plant were not detected in the combined-site analysis (Table G-18). Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no"). Although statistical differences were detected for the total number of first-position bolls at four sites (Table G-19), the assessed values of MON 88701 for the combined-site analysis were within their respective reference range. Therefore, the differences between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 3, answer "no").

G.14. Individual Site Environmental Interactions - Results and Discussion

G.14.1. Individual Site Environmental Interactions - Results and Discussion – MON 88701 Not Treated with Dicamba or Glufosinate Herbicides – Study 1

(Qualitative Data Assessment)

In an individual site assessment, no differences were observed between MON 88701 and the conventional control for any of the 169 comparisons for the assessed abiotic stressors, including compaction, drought/dry, flood, hail, heat, nutrient deficiency, wet soil/excess precipitation, and wind damage (Table G-20).

In an individual site assessment, no differences were observed between MON 88701 and the control for any of the 170 comparisons for the assessed diseases, including anthracnose, *Ascochyta* leaf blight, bacterial blight, boll rot, cotton leaf rust, damping off, *Fusarium* wilt, leaf spots, *Pythium*, reniform nematode, *Rhizoctonia*, root-knot nematode, *Thielaviopsis*, and *Verticillium* wilt (Table G-21)

In an individual site assessment, no differences were observed between MON 88701 and the control for any of the 159 comparisons for the assessed arthropod stressors, including aphids, beet armyworms, cut worms, fall armyworms, fleahoppers,

grasshoppers, heliothines, southern corn rootworm beetles, soybean loopers, spider mites, stink bugs, tarnished plant bugs, thrips, and white flies (Table G-22).

(Quantitative Data Assessment)

Thrips Damage

A total of 15 thrips damage comparisons (three observation events × 5 sites) were made between MON 88701 and the conventional control in the individual site analysis (Table G-23). Of these comparisons, no numerical differences were observed for 10 comparisons for which p-values could not be generated due to lack of variability in the data. Four of the remaining five comparisons were not significantly different. MON 88701 had significantly less damage from thrips in observation 3 at the ARPR site (0.1 vs. 0.3). However, there was no significant difference between MON 88701 and the conventional control for Observation 3 in the combined-site analysis (Table VII-8). Therefore, the lack of difference for 14 comparisons and the one site/observation difference between MON 88701 and the conventional control were not indicative of a consistent plant response and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no").

Heliothines Damage

A total of 40 heliothine damage comparisons (4 observations × 5 sites) were made between MON 88701 and the conventional control in the individual site analysis (Table G-24). Of these comparisons, no numerical differences were observed for three comparisons for which p-values could not be generated due to lack of variability in the data. For the remaining 37 comparisons, no statistically significant differences were detected between MON 88701 and the control for 35 out of 37 comparisons (Table G-24). Two statistically significant differences were detected between MON 88701 and the parental control. Plants of MON88701 had fewer heliothine damage fruiting bodies compared to the conventional control in Observation 4 at ARAU (8.7 vs. 15.1%), and more live larvae Observation 1 at GACH (0.5 vs. 0.1). Although the above statistical differences were detected, the assessed values of MON 88701 were not significantly different than the control in the combined-site analysis (Table VII-9). Therefore, the lack of difference for 38 comparisons and the two site/observation differences between MON 88701 and the conventional control were not indicative of a consistent plant response, and MON 88701 is unlikely to have increased plant pest/weed potential or an adverse environmental impact compared to the conventional control (See Figure VII-1, *Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods*, step 2, answer "no").

Arthropod Abundance

A total of 178 comparisons were made between MON 88701 and the conventional control for arthropod abundance involving the following pest- and beneficial-arthropods: aphids, cabbage loopers, fall armyworms, fleahoppers, heliothines, southern armyworms, stink bugs, tarnished plant bugs, thrips, white flies, big eyed bugs, braconids, Damsel bugs, lacewings, ladybird beetles, *Orius* spp and Araneae (spiders) (Tables G-25 and G-26). No statistically significant differences were detected between MON 88701 and the conventional control for 173 out of 178 comparisons, including 89 pest arthropod comparisons and 89 beneficial arthropod comparisons. The five differences detected between MON 88701 and the conventional control included two differences for pest arthropods and three differences for beneficial arthropods.

At collection 4 at LABU, the abundance of stink bugs per plot in MON 88701 was lower compared to the conventional control (0.3 vs. 1.8 per plot) and lower for tarnished plant bugs (0.5 vs. 2.0). For tarnished plant bugs, the mean abundance value for MON 88701 was within the reference ranges for the differences detected. For stink bugs, the mean abundance value for MON 88701 was outside the reference range for the difference detected. Since the two differences mentioned above were not consistently detected in multiple environments, these data support a conclusion that the differences are considered not biologically meaningful in terms of plant pest potential or an adverse environmental impact (See Section VII.B.1.1., Interpretation of Environmental Interactions Data).

The abundance of Damsel bugs per plot MON 88701 was higher compared to the conventional control in Collection 2 at GACH (6.0 vs. 2.3) and lower for *Orius* spp. in Collection 2 (0.0 vs. 1.5 per plot) and collection 3 (0.5 vs. 2.8 per plot) at the ARAU site. The mean abundance value for MON 88701 was within the reference range for the difference detected for Damsel bugs. The mean abundance values for *Orius* spp. in Collection 2 and collection 3 at the ARAU site were outside their respective reference range. However, the differences detected for *Orius* spp. were not consistently detected across collections or sites (Table G-26). Thus, the detected differences in beneficial arthropod abundance were not indicative of a consistent response associated with MON 88701 and are not considered biologically meaningful in terms of an adverse environmental impact of MON 88701 compared to conventional cotton (See Section VII.B.1.1., Interpretation of Environmental Interactions Data).

G.14.2. Individual Site Environmental Interactions - Results and Discussion – MON 88701 Not Treated with Dicamba or Glufosinate Herbicides – Study 2

In an individual site assessment, no differences were observed between MON 88701 and the control for any of the 127 comparisons for the assessed abiotic stressors, including compaction, drought, dry, flood, hail damage, heat, nutrient deficiency, wet soil, excess precipitation, and wind damage (Table G-27).

In an individual site assessment, no differences were observed between MON 88701 and the control for any of the 129 comparisons for the assessed diseases, including anthracnose, *Ascochyta* leaf blight, bacterial blight, boll rot, cotton leaf rust, damping

off, *Fusarium* wilt, leaf spots, *Pythium*, reniform nematode, *Rhizoctonia*, root-knot nematode, *Thielaviopsis*, and *Verticillium* wilt (Table G-28).

In an individual site assessment, no differences were observed between MON 88701 and the control for any of the 129 comparisons for the assessed arthropod stressors, including aphids, beet armyworms, cabbage loopers, cut worms, fall armyworms, fleahoppers, grasshoppers, heliothines, southern corn rootworm beetle, soybean loopers, spider mites, stink bugs, tarnished plant bugs, thrips, and white flies (Table G-29).

These results support a conclusion that MON 88701 would not confer a plant pest risk or significant environmental impact compared to conventional cotton (See Section VII.B.1.1., Interpretation of Environmental Interactions Data).

Table G-5. Study 1 Data Missing or Excluded from Analysis

Site	Material name	Material type	Plots	Characteristics	Reason for exclusion
AZME	All	All	All	All	Site was dropped due to low stand count in the plots, poor trial maintenance, and non-reliable data collection by the cooperator.
All	All	All	All	Nodes above cracked boll observations 1, 2, and 3	Nodes above white flower data were sufficient in providing growth and development trend relevant for showing crop advancement towards cutout.
ARAU and LABU	All	All	All	Arthropod collection 1	Neonate soybean looper identification was questionable because of the size.
LABU	All	All	All	Arthropod collection 3	Beneficial species from collection 2 were recorded for collection 3 as there were no beneficial insects in collection 3
KSLA	All	All	All	14 D stand counts	Stand counts taken on 6/16 were dropped due to low count and were taken again on 6/23 from all four rows of each plot. Then rows with good count were chosen for data collection.
KSLA	All	All	All	14 D plant vigor	Vigor was taken at 6/16 and 6/23 but 6/23 data were dropped because it was taken 21 days after planting.
NCBD	Coker 130	Control	403	Final stand count	Stand count was poor in these plots as couple feet in the plot were destroyed by the lightning strike.
NCBD	Coker 130	Control	403	Seed cotton yield	A couple feet in the plot were destroyed by a lightening strike.
NCME	DP 435 and DP 5415	Reference	206, 305, 406	Stand count at 30 DAP and final stand count	Stand count was poor in these plots because of poor germplasm.
NCME	DP 5415	Reference	406	Seedcotton yield	Yield was very low due to poor stand count.
TXPL	All	All	All	Nodes above cracked boll observations 4	This was extra collection and not needed.

Table G-6. Study 2 Data Missing or Excluded from Analysis

Site	Material name	Material type	Plots	Characteristics	Reason for exclusion
ARTI	SG125	Reference	106	Plant mapping	Plant mapping data sheet was misplaced after collection.
TXPO	All	All	All	All	Plot area was destroyed by lightning.
ALL	All	All	All	Nodes above cracked boll and days of planning to first cracked boll date	Nodes above white flower data were sufficient in providing growth and development trends relevant for showing crop advancement towards cutout. Repetition of summary would not have added any value.
ALL	All	All	All	Days of planting to first flower date	Data on three observations on nodes above white flower was sufficient in proving data on crop growth and development. Repetition of summary would not have added any value.
ARTI GACH LACH NCME	FM 989	Reference	All	ALL	Due to poor germination and stand establishment, reference FM 989 will be excluded from all phenotypic data analysis and reporting. Germination ranged from 9.5 to 40.5 % of the target stand counts.

Table G-7. Study 1 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

	Phenotypic Characteristics									
	Stand count at 14 DAP (# per ~6 m)		Stand count at 30 DAP (# per ~6 m)		Final stand count		Plant vigor at 14 DAP[1]		Plant vigor at 30 DAP[2]	
Site	MON 88701 (SE)[3]	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701	Control	MON 88701	Control
ARAU	165.7 (10.0)	182.2 (12.0)	159.3 (7.3)	174.0 (11.1)	154.2 (9.0)	181.0 (4.9)	3.0-4.0	2.0-3.0	2.0-3.0	2.0-3.0
ARPR	179.8 (3.2)	185.3 (4.1)	179.7 (3.2)	183.5 (4.7)	178.3 (3.5)	181.8 (4.7)	5.0-6.0	6.0-6.0	4.0-4.0	2.0-3.0
ARTI	173.0 (6.5)	175.5 (5.0)	171.5 (6.4)	173.5 (5.3)	171.0 (6.4)	172.5 (4.9)	1.0-1.0	1.0-1.0	1.0-1.0	1.0-1.0
GACH	170.7 (2.7)	171.7 (4.0)	168.8 (2.3)	167.7 (3.9)	166.8 (2.9)	168.5 (3.6)	3.0-4.0	3.0-4.0	2.0-5.0	2.0-4.0
GAJE	178.8 (3.3)	182.8 (5.8)	168.5 (4.9)	160.8 (3.3)	167.5 (1.8)*	156.3 (4.9)	1.0-2.0	1.0-1.0	2.0-2.0	2.0-2.0
KSLA	127.8 (2.8)	141.5 (3.2)	74.5 (2.5)	74.0 (0.7)	70.3 (2.3)	70.3 (0.8)	2.0-2.0	2.0-2.0	2.0-2.0	2.0-2.0
LABU	147.5 (1.0)	155.7 (3.3)	146.2 (1.3)	160.0 (4.1)	94.3 (1.8)	91.8 (0.7)	1.0-3.0	2.0-3.0	1.0-2.0	1.0-2.0
LACH	100.8 (3.0)*	127.8 (1.1)	101.8 (2.6)*	125.0 (2.6)	63.3 (0.9)*	73.5 (4.7)	2.0-3.0	2.0-3.0	2.0-2.0	1.0-2.0
NCBD	117.3 (2.3)	125.3 (6.8)	106.8 (5.0)	116.5 (7.5)	106.5 (2.7)	115.3 (8.8)	3.0-3.0	2.0-3.0	3.0-4.0	2.0-3.0
NCME	95.8 (22.8)	105.0 (11.8)	90.5 (23.0)	99.8 (12.3)	90.5 (21.6)	97.0 (12.2)	3.0-6.0	3.0-6.0	3.0-5.0	3.0-5.0
NMGA	116.5 (17.3)	116.0 (12.8)	70.0 (0.0)	70.0 (0.0)	70.3 (0.5)	70.8 (0.6)	1.0-2.0	2.0-2.0	1.0-1.0	1.0-1.0
NMLC	122.5 (6.7)	116.0 (18.8)	70.0 (0.0)	70.0 (0.0)	70.8 (0.9)	71.8 (1.0)	2.0-2.0	1.0-2.0	1.0-1.0	1.0-1.0
SCEK	182.7 (4.3)	188.2 (3.2)	183.3 (3.4)	189.3 (3.1)	179.2 (2.6)	181.3 (3.1)	2.0-3.0	2.0-3.0	2.0-4.0	2.0-3.0
TXPL	163.0 (3.8)	151.0 (15.9)	142.8 (8.3)	155.5 (9.5)	157.8 (1.9)	146.8 (13.9)	1.0-1.0	1.0-1.0	3.0-3.0	3.0-3.0
TXPO	147.8 (13.3)	162.3 (12.1)	143.3 (9.1)	145.5 (13.0)	137.0 (6.1)	151.0 (5.4)	1.0-1.0	1.0-1.0	1.0-1.0	1.0-1.0

Table G-7. Study 1 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic Characteristics									
	Plant height at 30 DAP (cm)		Plant height before harvest (cm)		Nodes above white flower (obs. 1)		Nodes above white flower (obs. 2)		Nodes above white flower (obs. 3)	
Site	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)
ARAU	30.9 (0.5)	33.7 (1.2)	159.3 (2.9)	168.8 (8.8)	7.7 (0.1)	7.6 (0.2)	7.1 (0.3)	7.1 (0.1)	6.7 (0.5)	6.5 (0.2)
ARPR	29.1 (1.5)	31.8 (2.6)	126.9 (3.6)	134.4 (3.1)	7.7 (0.2)	7.2 (0.2)	7.1 (0.1)*	6.6 (0.2)	6.1 (0.3)	5.5 (0.2)
ARTI	29.5 (0.6)	29.4 (0.3)	131.9 (2.9)	139.2 (4.8)	8.5 (0.1)	8.4 (0.1)	7.5 (0.2)	7.1 (0.2)	4.7 (0.1)	4.8 (0.1)
GACH	28.2 (0.4)	29.7 (1.4)	84.6 (2.6)	88.1 (2.0)	5.2 (0.1)	5.0 (0.2)	3.4 (0.2)	3.1 (0.3)	1.7 (0.1)	1.3 (0.2)
GAJE	27.0 (0.8)	27.9 (0.7)	87.1 (3.7)*	98.6 (5.3)	7.3 (0.6)	7.7 (0.5)	6.0 (0.2)	5.9 (0.1)	3.9 (0.0)	4.0 (0.1)
KSLA	17.5 (1.4)	19.4 (0.8)	129.9 (3.5)*	142.0 (5.5)	5.1 (0.2)*	4.4 (0.2)	4.2 (0.5)*	3.2 (0.2)	4.6 (0.5)*	3.5 (0.2)
LABU	15.8 (0.8)	17.2 (0.7)	150.2 (3.7)	161.2 (6.4)	7.5 (0.2)	7.6 (0.4)	7.0 (0.2)	6.6 (0.2)	7.5 (0.1)	7.5 (0.2)
LACH	12.4 (0.6)*	14.5 (0.6)	120.5 (3.2)*	140.5 (9.8)	8.2 (0.2)	8.5 (0.1)	7.8 (0.1)	7.6 (0.1)	6.9 (0.1)	7.0 (0.1)
NCBD	20.0 (1.8)*	24.5 (1.0)	104.8 (9.5)*	111.0 (10.5)	6.2 (0.5)	5.6 (0.5)	4.0 (0.4)	3.7 (0.6)	2.9 (0.5)	2.4 (0.6)
NCME	16.2 (1.1)	15.7 (0.3)	90.3 (4.4)	94.7 (2.1)	4.8 (0.1)	4.8 (0.1)	3.1 (0.1)	2.9 (0.1)	2.7 (0.3)	2.2 (0.3)
NMGA	5.0 (0.2)	5.2 (0.4)	92.3 (3.0)	96.6 (3.8)	8.6 (0.4)	8.4 (0.5)	8.7 (0.2)	8.5 (0.3)	5.9 (0.9)	6.1 (0.9)
NMLC	6.7 (0.7)*	8.3 (0.6)	96.0 (4.6)	94.6 (2.1)	8.4 (0.5)*	7.4 (0.3)	8.2 (0.3)	8.0 (0.2)	6.5 (0.4)	6.2 (0.3)
SCEK	8.0 (0.9)	8.4 (1.5)	98.9 (8.0)	97.8 (5.0)	5.3 (0.2)	5.0 (0.3)	5.4 (0.4)	4.9 (0.3)	5.2 (0.3)*	4.6 (0.1)
TXPL	8.0 (0.4)	8.1 (0.3)	44.9 (0.7)*	48.4 (0.8)	5.0 (0.1)*	4.5 (0.2)	4.4 (0.2)	3.9 (0.2)	2.0 (0.1)	1.8 (0.1)
TXPO	19.9 (0.8)	22.6 (0.8)	129.7 (1.2)	129.9 (0.9)	8.8 (0.1)	8.5 (0.3)	7.0 (0.2)	6.6 (0.1)	6.4 (0.3)*	5.7 (0.2)

Table G-7. Study 1 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic Characteristics	
	Seed cotton yield (Kg/ha)	
Site	MON 88701 (SE)	Control (SE)
ARAU	2912.9 (231.8)*	2166.8 (250.2)
ARPR	3452.4 (202.0)	3309.7 (155.9)
ARTI	3444.8 (90.4)	3488.7 (87.3)
GACH	2880.8 (97.8)	2875.6 (101.1)
GAJE	2320.4 (42.3)*	1843.1 (256.8)
KSLA	4330.4 (184.1)	4341.9(365.1)
LABU	1534.1 (153.7)	1719.4 (67.8)
LACH	1464.1 (245.0)	1736.0 (87.4)
NCBD	3990.5 (375.5)	4112.1 (299.2)
NCME	1324.7 (110.5)	1296.3 (140.9)
NMGA	3459.6 (211.1)	3498.9 (165.2)
NMLC	4771.7 (199.9)	4522.5 (158.1)
SCEK	3625.0 (125.2)	3901.9 (143.5)
TXPL	3638.4 (114.1)	3689.3 (29.6)
TXPO	916.5 (115.9)	856.8 (41.5)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and conventional control (n = 4).
[1]Plant vigor score range (minimum-maximum). The range of plant vigor score at 14 DAP for the references is as follows: ARAU 3-5; ARPR 3-6; ARTI 1-2; GACH 3-7; GAJE 1-3; KSLA 2-4; LABU 2-5; LACH 2-5; NCBD 3-4; NCME 3-6; NMGA 1-3; NMLC 2-3; SCEK 2-4; TXPL 1-1; TXPO 1-1. Plant vigor was rated per plot using a rating scale of 1-9 where: 1-3 is excellent vigor, 4-6 is average vigor, and 7-9 is poor vigor.
[2]Plant vigor score range (minimum-maximum). The range of plant vigor score at 30 DAP for the references is as follows: ARAU 3-5; ARPR 4-7; ARTI 1-1; GACH 3-7; GAJE 2-2; KSLA 2-3; LABU 2-3; LACH 1-4; NCBD 2-4; NCME 3-7; NMGA 1-5; NMLC 1-5; SCEK 3-4; TXPL 3-3; TXPO 1-1.
[3] SE = Standard error

Table G-8. Study 1 - Individual Site Phenotypic Comparison – Seed Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control.

	Seed index (g per 100 fuzzy seed)		Total seed per boll (# per boll)		Mature seed per boll (# per boll)		Immature seed per boll (# per boll)	
Site	MON 88701 (SE)[1]	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)
ARAU	10.6 (0.4)	10.6 (0.2)	31.6 (0.3)*	29.6 (1.0)	20.5 (1.2)	19.2 (0.9)	11.1 (1.4)	10.4 (0.8)
ARPR	9.4 (0.2)*	10.3 (0.1)	29.4 (0.3)*	27.3 (0.9)	27.3 (0.6)*	21.7 (0.7)	2.1 (0.3)*	5.7 (1.0)
ARTI	10.1 (0.2)*	10.9 (0.1)	26.0 (0.7)	26.6 (0.5)	20.2 (1.0)*	17.6 (0.4)	5.7 (1.2)*	9.0 (0.5)
GACH	10.3 (0.6)	10.4 (0.5)	28.0 (0.9)	27.6 (0.7)	17.4 (0.5)	17.0 (0.8)	10.6 (1.3)	10.6 (1.3)
GAJE	8.3 (0.4)*	9.8 (0.2)	25.8 (0.9)	26.3 (1.3)	15.6 (0.8)	15.2 (2.0)	10.2 (0.2)	11.1 (0.7)
KSLA	11.8 (0.2)*	12.5 (0.2)	31.2 (1.1)*	28.1 (0.4)	29.9 (1.1)*	26.6 (0.4)	1.3 (0.1)	1.5 (0.1)
LABU	9.4 (0.2)*	10.5 (0.1)	29.5 (0.4)	27.9 (0.7)	21.2 (0.8)	18.9 (1.1)	8.4 (1.0)	9.0 (1.4)
LACH	8.8 (0.3)*	9.8 (0.3)	28.4 (0.8)	28.3 (0.7)	15.5 (1.2)	16.0 (0.7)	12.9 (0.8)	12.4 (0.5)
NCBD	9.0 (0.1)*	9.8 (0.3)	30.1 (1.5)	27.8 (1.7)	21.1 (3.5)	16.2 (2.1)	9.0 (2.5)	11.6 (1.0)
NCME	9.7 (0.2)*	10.6 (0.1)	31.7 (2.0)*	27.5 (0.8)	28.4 (1.0)*	22.1 (0.7)	3.4 (1.0)	5.4 (1.0)
NMGA	10.6 (0.2)*	11.4 (0.2)	34.4 (0.4)*	30.3 (0.8)	32.9 (0.6)*	28.8 (0.6)	1.6 (0.3)	1.5 (0.3)
NMLC	10.5 (1.0)	10.5 (0.6)	31.0 (0.7)*	25.9 (1.5)	26.5 (1.0)*	21.6 (1.3)	4.5 (0.6)	4.3 (0.5)
SCEK	8.5 (0.2)	9.3 (0.2)	27.1 (0.9)	27.1 (0.8)	20.5 (0.8)	16.0 (2.1)	6.5 (0.5)*	11.1 (2.1)
TXPL	11.5 (0.3)	11.5 (0.6)	28.3 (0.6)	28.0 (0.8)	25.4 (0.7)	24.5 (0.8)	2.9 (0.6)	3.5 (0.8)
TXPO	9.0 (0.2)	9.6 (0.4)	23.3 (0.2)	22.7 (0.7)	16.8 (0.8)	14.7 (0.9)	6.6 (0.7)	8.0 (0.6)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and conventional control (n = 4).
[1] SE = Standard error

Table G-9. Study 1 - Individual Site Phenotypic Comparison – Boll and Fiber Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

	Boll weight (g/boll)		Micronaire (mic units)[1]		Elongation (%)		Strength (g/tex)		Uniformity (%)		Length (cm)	
Site	MON 88701 (SE)[2]	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)	MON 88701 (SE)	Control (SE)
ARAU	4.9 (0.1)	4.9 (0.2)	3.8 (0.0)	3.6 (0.1)	6.8 (0.2)*	6.2 (0.1)	33.2 (0.5)	32.3 (0.2)	85.3 (0.1)	84.4 (0.3)	3.0 (0.0)	3.0 (0.0)
ARPR	4.8 (0.1)	4.6 (0.2)	5.2 (0.1)	5.0 (0.2)	5.4 (0.2)	5.2 (0.4)	29.8 (0.3)	29.8 (0.6)	83.0 (0.4)	82.4 (0.4)	2.7 (0.0)	2.7 (0.1)
ARTI	4.1 (0.1)*	4.8 (0.1)	4.5 (0.1)	4.7 (0.0)	5.2 (0.2)	5.5 (0.3)	32.1 (0.1)	31.0 (0.5)	84.4 (0.4)	83.6 (0.1)	2.9 (0.0)	2.8 (0.0)
GACH	4.2 (0.1)*	4.5 (0.1)	4.6 (0.0)	4.6 (0.1)	7.1 (0.1)	6.8 (0.3)	30.8 (0.5)	30.6 (0.5)	83.8 (0.2)	84.1 (0.5)	2.8 (0.0)	2.9 (0.0)
GAJE	3.6 (0.1)	4.0 (0.3)	3.9 (0.1)	3.7 (0.1)	7.1 (0.5)	6.7 (0.3)	32.2 (0.7)	32.1 (0.2)	84.0 (0.2)	83.8 (0.2)	2.8 (0.0)	2.9 (0.0)
KSLA	6.7 (0.2)	6.3 (0.1)	4.5 (0.0)	4.5 (0.1)	5.8 (0.3)	5.6 (0.4)	31.5 (0.4)*	30.3 (0.3)	84.1 (0.5)	83.4 (0.3)	3.0 (0.0)	3.0 (0.0)
LABU	4.5 (0.1)	4.7 (0.1)	4.7 (0.1)	4.7 (0.1)	5.0 (0.3)	5.4 (0.3)	32.3 (0.6)	31.2 (0.3)	84.1 (0.1)	84.1 (0.2)	2.8 (0.1)	2.9 (0.0)
LACH	4.1 (0.2)	4.5 (0.2)	4.4 (0.0)	4.5 (0.0)	5.5 (0.2)	6.0 (0.4)	30.5 (0.2)	29.9 (0.2)	83.7 (0.3)	83.3 (0.4)	2.8 (0.0)	2.8 (0.0)
NCBD	5.4 (0.2)	5.1 (0.4)	4.7 (0.0)	4.5 (0.5)	5.7 (0.3)*	6.6 (0.4)	32.6 (1.1)*	30.9 (0.8)	85.1 (0.4)	84.7 (0.4)	2.9 (0.1)	2.9 (0.1)
NCME	5.7 (0.3)	5.3 (0.1)	5.0 (0.0)	4.8 (0.1)	6.2 (0.1)	6.4 (0.3)	32.6 (0.2)	31.8 (0.3)	84.1 (0.4)	84.2 (0.3)	2.8 (0.0)*	2.9 (0.0)
NMGA	5.9 (0.1)	5.8 (0.1)	5.1 (0.1)*	4.9 (0.0)	5.9 (0.2)	6.0 (0.2)	30.9 (0.2)	31.2 (0.4)	82.3 (0.8)	83.7 (0.2)	2.8 (0.0)	2.9 (0.0)
NMLC	5.1 (0.1)*	4.6 (0.3)	4.7 (0.1)	4.6 (0.1)	7.1 (0.2)	6.8 (0.2)	31.2 (0.3)*	30.0 (0.2)	83.5 (0.3)	83.0 (0.4)	2.9 (0.0)	2.9 (0.0)
SCEK	4.1 (0.2)	4.4 (0.2)	4.7 (0.1)	4.7 (0.1)	5.1 (0.1)	5.2 (0.1)	32.1 (0.5)	31.2 (0.3)	83.3 (0.2)	83.7 (0.1)	2.8 (0.0)	2.8 (0.0)
TXPL	5.8 (0.1)	6.0 (0.1)	4.9 (0.1)*	4.5 (0.1)	7.2 (0.1)	7.3 (0.3)	34.1(0.1)*	32.2 (0.5)	85.4 (0.4)*	84.1 (0.4)	2.9 (0.0)	2.9 (0.0)
TXPO	3.3 (0.1)	3.2 (0.2)	4.6 (0.1)	4.5 (0.1)	5.0 (0.4)	4.8 (0.1)	31.0 (0.6)	31.2 (0.2)	83.8 (0.6)	83.2 (0.2)	2.7 (0.0)	2.8 (0.0)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and conventional control (n = 4).
[1] Measure of fiber fineness and maturity (expressed in dimensionless micronaire units).
[2] SE = Standard error.

Table G-10. Study 2 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

	Phenotypic Characteristic (units)									
	Stand count 14 DAP[1] (# per plot)		Stand count at 30 DAP (# per plot)		Final Stand Count at harvest		Plant Height at 30 DAP (cm)		Plant Height before harvest (cm)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE)[2]	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	185.5 (1.9)	179.8 (2.3)	180.3 (0.9)	179.5 (2.9)	178.0 (0.4)	177.5 (3.5)	20.5 (0.6)	21.1 (0.5)	71.7 (5.0)	73.5 (5.1)
ARTI	155.3 (5.9)	164.8 (7.2)	153.8 (6.3)	164.3 (7.1)	153.3 (6.5)	163.8 (7.2)	33.0 (0.2)	32.8 (0.2)	128.1 (3.0)	126.9 (3.1)
GACH	131.3 (8.4)	132.8 (8.0)	129.0 (8.0)	127.8 (10.3)	128.5 (9.4)	129.5 (9.1)	25.1 (1.0)	25.8 (0.8)	100.2 (4.5)	110.2 (8.7)
GAJE	179.8 (4.4)	181.3 (2.7)	166.0 (2.3)	164.0 (3.0)	162.3 (6.0)	157.3 (8.4)	23.6 (1.1)	26.0 (0.7)	77.4 (4.4)*	92.6 (7.9)
KSLA	156.5 (7.4)	162.5 (5.9)	154.8 (7.4)	158.5 (5.3)	150.5 (7.0)	150.0 (6.7)	15.7 (0.6)*	18.3 (1.2)	113.5 (3.0)*	127.0 (3.3)
LACH	98.8 (3.1)	110.3 (5.4)	101.0 (3.1)	112.5 (7.6)	92.3 (1.1)	94.8 (2.9)	13.5 (0.1)*	15.9 (0.8)	154.4 (5.7)*	180.0 (3.1)
NCBD	141.8 (2.3)	140.3 (3.2)	140.8 (2.2)	140.3 (3.6)	132.5 (2.5)	134.3 (2.3)	17.8 (0.9)	19.4 (0.7)	80.3 (2.1)	84.7 (4.8)
NCME	132.0 (8.2)	148.0 (19.5)	158.5 (6.3)	160.3 (9.7)	146.3 (9.0)	166.3 (8.3)	17.2 (0.7)*	19.7 (0.9)	71.4 (5.2)*	85.0 (3.4)
NMLC	157.5 (7.5)	168.8 (11.9)	154.5 (7.3)	164.0 (2.4)	153.3 (6.2)	163.3 (5.8)	7.0 (0.2)	7.2 (0.2)	96.7 (2.4)	102.7 (1.9)
SCEK	186.3 (3.7)	191.5 (4.6)	182.5 (3.6)	190.3 (3.9)	181.5 (3.0)	188.8 (3.8)	16.7 (1.1)	17.9 (1.3)	104.7 (3.6)	111.7 (1.4)
TXPL	130.8 (3.3)	125.0 (10.4)	122.3 (7.4)	119.0 (8.8)	130.5 (3.2)	130.0 (11.3)	7.8 (0.6)	7.4 (0.5)	58.6 (1.0)	60.9 (1.3)

Table G-10. Study 2 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic Characteristic (units)							
	1st Vigor[3,4] rating		2nd Vigor[3,5] rating		Nodes above white flower (obs. 1)		Nodes above white flower (obs. 2)	
Site	MON 88701 Range	Control Range	MON 88701 Range	Control Range	MON 88701 Mean (SE)	Control Mean (SE)	MON 88701 Mean (SE)	Control Mean (SE)
ARPR	4 – 6	3 - 4	4 - 5	3 - 5	5.5 (0.1)	5.7 (0.2)	3.7 (0.2)	3.6 (0.2)
ARTI	2 – 3	2 - 3	2 - 2	2 - 2	8.9 (0.3)	8.5 (0.2)	6.9 (0.3)	6.5 (0.2)
GACH	3 – 4	2 - 5	3 - 5	4 - 6	5.9 (0.2)*	5.3 (0.2)	4.7 (0.1)	4.1 (0.4)
GAJE	1 – 2	1 - 1	2 - 2	2 - 2	6.3 (0.3)	6.6 (0.5)	5.9 (0.1)	5.7 (0.2)
KSLA	2 – 5	2 - 3	2 - 3	2 - 2	4.8 (0.3)	4.6 (0.2)	4.4 (0.7)	4.2 (0.6)
LACH	4 – 7	3 - 5	2 - 3	1 - 2	7.9 (0.2)	7.6 (0.1)	8.2 (0.2)	8.4 (0.1)
NCBD	2 – 2	2 - 3	3 - 3	3 - 3	7.2 (0.1)	7.1 (0.5)	4.8 (0.2)*	3.9 (0.2)
NCME	2 – 5	1 - 5	2 - 3	2 - 3	5.1 (0.3)	5.2 (0.2)	3.2 (0.1)	3.3 (0.2)
NMLC	1 – 3	2 - 2	1 - 2	1 - 1	7.4 (0.3)	7.1 (0.2)	8.1 (0.1)	8.1 (0.3)
SCEK	2 – 4	3 - 6	2 - 3	3 - 3	7.5 (0.2)	7.4 (0.2)	4.8 (0.1)	4.5 (0.1)
TXPL	1 -1	1 - 1	3 - 3	3 - 3	5.9 (0.2)	5.7 (0.1)	5.5 (0.2)*	5.1 (0.1)

Table G-10. Study 2 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic characteristics (units)			
	Nodes above white flower (obs. 3)		Seedcotton yield (kg/ha)	
	MON 88701	Control	MON 88701	Control
Site	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	2.3 (0.2)	2.4 (0.4)	2,561.6 (191.4)	2,301.7 (173.4)
ARTI	4.8 (0.1)	4.7 (0.1)	3,864.4 (250.8)	3,869.0 (112.9)
GACH	4.1 (0.1)*	3.6 (0.3)	4,424.4 (74.0)	4,471.5 (99.5)
GAJE	3.9 (0.1)	3.8 (0.0)	1,750.1 (261.1)	1,548.4 (342.4)
KSLA	3.7 (0.4)*	2.5 (0.4)	4,487.0 (294.6)*	3,726.5 (81.7)
LACH	5.8 (0.1)	5.7 (0.1)	1,956.0 (173.0)	2,046.9 (210.4)
NCBD	3.4 (0.3)	3.0 (0.3)	4,224.0 (326.0)	3,792.7 (283.0)
NCME	2.0 (0.2)	1.9 (0.1)	1,508.4 (112.7)	1,610.6 (206.9)
NMLC	7.5 (0.2)	7.1 (0.2)	1,938.4 (22.1)*	1,479.3 (65.6)
SCEK	3.8 (0.3)	3.5 (0.1)	5,219.8 (236.4)	5,424.3 (148.4)
TXPL	3.6 (0.1)	3.5 (0.3)	4,741.3 (165.8)	4,534.5 (215.6)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and control (n = 4).
[1] DAP = Days after planting.
[2] SE = Standard error.
[3] Plant vigor was rated per plot using a rating scale of 1-9 where: 1-3 is excellent vigor, 4-6 is average vigor, and 7-9 is poor vigor.
[4] First plant vigor score range (minimum-maximum). The range of plant vigor score at 14 DAP for the references is as follows: ARPR 3-6; ARTI 2-3; GACH 4-6; GAJE 1-3; KSLA 2-5; LACH 4-7; NCBD 3-3; NCME 1-6; NMLC 2-5;SCEK 4-6; TXPL 1-1.
[5] Plant vigor score range (minimum-maximum). The range of plant vigor score at 30 DAP for the references is as follows: ARPR 4-6; ARTI 2-2; GACH 3-6; GAJE 2-2; KSLA 2-3; LACH 2-3; NCBD 3-3; NCME 2-3; NMLC 1-3; SCEK 2-3; TXPL 3-3.

Table G-11. Study 2 - Individual Site Phenotypic Comparison – Seed Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

	Seed Characteristics							
	Seed index (g per 100 seed)		Total seed per boll (# per boll)		Mature seed per boll (# per boll)		Immature seed per boll (# per boll)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE) [1]	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	8.8 (0.2)*	10.0 (0.4)	26.8 (0.4)*	23.9 (0.7)	24.2 (0.4)*	15.9 (0.6)	2.6 (0.2)*	8.0 (0.6)
ARTI	9.9 (0.3)*	12.0 (1.0)	28.4 (0.3)	26.8 (0.7)	23.7 (0.7)*	18.0 (0.6)	4.6 (0.6)*	8.9 (1.2)
GACH	9.1 (0.3)	9.7 (0.3)	29.4 (0.7)	27.4 (1.1)	24.9 (1.0)	23.4 (1.1)	4.5 (1.4)	4.0 (0.6)
GAJE	8.5 (0.4)*	10.4 (0.5)	26.6 (1.1)	24.6 (0.7)	15.0 (0.5)*	11.6 (0.1)	11.6 (0.8)	13.0 (0.8)
KSLA	11.7 (0.2)*	12.8 (0.3)	28.4 (1.7)*	24.9 (0.4)	25.9 (1.9)*	22.7 (1.0)	2.5 (0.6)	2.2 (0.6)
LACH	9.4 (0.2)*	10.3 (0.2)	31.4 (0.4)	28.7 (1.2)	20.7 (1.6)	17.5 (1.9)	10.7 (1.8)	11.2 (0.8)
NCBD	8.8 (0.2)*	10.5 (0.2)	27.0 (0.8)	25.7 (1.3)	18.4 (1.2)	18.2 (0.9)	8.6 (0.6)	7.5 (0.5)
NCME	8.8 (0.1)*	10.2 (0.1)	28.3 (0.5)	27.6 (1.7)	25.8 (0.6)	23.0 (1.7)	2.5 (0.7)	4.6 (0.2)
NMLC	10.3 (0.2)	10.9 (0.2)	33.8 (2.0)*	30.7 (1.1)	30.7 (2.2)	28.1 (1.4)	3.2 (1.0)	2.6 (0.4)
SCEK	8.5 (0.2)*	9.8 (0.2)	28.9 (2.0)	27.3 (1.3)	20.8 (1.4)	19.1 (1.4)	8.1 (2.0)	8.2 (1.2)
TXPL	9.9 (0.3)*	11.0 (0.3)	31.4 (0.6)	30.1 (0.9)	26.7 (0.8)	25.7 (0.7)	4.7 (0.8)	4.4 (1.1)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and control (n = 0.4).
[1] SE = Standard error.

Table G-12. Study 2 - Individual Site Phenotypic Comparison – Boll and Fiber Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

	Weight per Boll (g/boll)		Fiber Micronaire (mic units)[2]		Fiber Elongation (%)		Fiber Strength (g/tex)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE)[1]	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	4.3 (0.1)	4.1 (0.2)	5.0 (0.1)	4.9 (0.1)	5.0 (0.3)*	5.7 (0.2)	30.8 (0.4)	30.5 (0.4)
ARTI	4.5 (0.0)	4.4 (0.1)	5.0 (0.2)	4.8 (0.1)	5.4 (0.2)	5.4 (0.2)	29.7 (0.3)	28.8 (0.4)
GACH	5.0 (0.2)	4.9 (0.1)	4.8 (0.1)	4.7 (0.1)	5.9 (0.1)	6.3 (0.3)	30.4 (0.4)	29.5 (0.5)
GAJE	3.6 (0.2)	3.7 (0.1)	3.9 (0.1)	3.6 (0.1)	7.5 (0.5)	7.0 (0.3)	31.1 (0.6)	31.5 (0.5)
KSLA	5.8 (0.3) *	5.3 (0.1)	4.3 (0.0)	4.4 (0.1)	6.3 (0.2)	6.2 (0.3)	30.4 (0.1)*	29.4 (0.3)
LACH	5.4 (0.1)	5.4 (0.3)	4.7 (0.1)	4.6 (0.1)	5.5 (0.3)	5.9 (0.3)	30.7 (0.6)	29.9 (0.2)
NCBD	4.6 (0.3)	4.7 (0.2)	4.4 (0.1)	4.3 (0.1)	5.9 (0.1)	6.0 (0.2)	32.6 (0.9)	32.7 (0.4)
NCME	4.9 (0.1)	5.0 (0.3)	4.9 (0.1)	4.9 (0.1)	6.0 (0.3)	5.9 (0.4)	31.8 (0.2)	31.2 (0.3)
NMLC	5.7 (0.3)*	5.1 (0.3)	4.9 (0.0)*	4.7 (0.1)	6.2 (0.1)	6.4 (0.2)	30.1 (0.3)*	27.3 (0.5)
SCEK	4.6 (0.3)	4.4 (0.4)	4.9 (0.0)	4.9 (0.0)	7.0 (0.2)	6.9 (0.2)	29.9 (0.4)	30.0 (0.4)
TXPL	5.6 (0.1)	5.7 (0.1)	4.6 (0.1)	4.6 (0.1)	6.6 (0.2)	6.5 (0.2)	32.0 (0.3)*	31.0 (0.3)

Table G-12. Study 2 - Individual Site Phenotypic Comparison – Boll and Fiber Characteristics - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

	Fiber Uniformity (%)		Fiber Length (cm)	
	MON 88701	Control	MON 88701	Control
Site	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	83.9 (0.2)	84.6 (0.1)	2.8 (0.0)*	2.9 (0.0)
ARTI	83.6 (0.4)	83.6 (0.1)	2.7 (0.0)	2.8 (0.0)
GACH	83.6 (0.4)	83.4 (0.4)	2.8 (0.0)	2.8 (0.0)
GAJE	82.6 (0.9)	82.5 (0.6)	2.8 (0.1)	2.8 (0.1)
KSLA	84.2 (0.2)*	83.3 (0.1)	3.1 (0.0)	3.0 (0.0)
LACH	84.1 (0.3)	83.4 (0.4)	2.8 (0.0)	2.8 (0.0)
NCBD	84.8 (0.2)	84.8 (0.6)	2.8 (0.0)*	2.9 (0.0)
NCME	82.6 (0.5)	83.2 (0.6)	2.7 (0.0)	2.8 (0.1)
NMLC	83.4 (0.6)*	81.1 (0.7)	2.8 (0.0)	2.7 (0.1)
SCEK	83.1 (0.2)	83.9 (0.1)	2.7 (0.0)	2.8 (0.0)
TXPL	83.5 (0.3)	83.9 (0.3)	2.8 (0.0)	2.8 (0.0)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and control (n = 4).
[1] SE = Standard error.
[2] Measure of fiber fineness and maturity (expressed in dimensionless micronaire units).

Table G-13. Study 2 - Combined-Site Phenotypic Comparison - Growth and Development Characteristics - of MON 88701 Treated with Dicamba and Glufosinate Herbicides Compared to the Conventional Control

Phenotypic Characteristic (units)	MON 88701 Mean (SE)[2]	Control Mean (SE)	Reference Range[1] Minimum	Reference Range[1] Maximum
Stand Count at 14 DAP[3] (# per plot)	152.5 (4.9)	155.0 (4.4)	108.4	135.8
Stand Count at 30 DAP (# per plot)	151.1 (4.8)	152.8 (4.0)	105.8	134.1
Final Stand Count at harvest (# per plot)	147.7 (5.0)	150.5 (4.3)	110.5	137.7
Plant Height at 30 DAP (cm)	18.1 (1.1)*	19.2 (1.1)	11.4	20.7
Plant Height at harvest (cm)	98.4 (4.4)*	105.0 (4.9)	85.2	121.9
Nodes Above White Flower: (# of nodes at observation 1)	6.7 (0.2)*	6.4 (0.2)	6.0	7.3
(# of nodes at observation 2)	5.6 (0.3)*	5.2 (0.3)	4.8	5.7
(# of nodes at observation 3)	4.0 (0.2)	3.8 (0.2)	3.2	4.6
Seedcotton Yield (kg/ha)	3,295.5 (191.0)	3,164.1 (210.8)	2,181.7	3,970.8
Seed Index (g per 100 seed)	9.5 (0.2)*	10.7 (0.2)	9.4	12.4
Total Seed per Boll (# per boll)	28.5 (0.4)*	27.0 (0.4)	26.1	30.7
Mature Seed per Boll (# per boll)	22.8 (0.7)*	20.1 (0.8)	14.6	27.0
Immature Seed per Boll (# per boll)	5.7 (0.6)	6.9 (0.6)	2.7	14.4
Weight per Boll (g)	4.7 (0.1)	4.8 (0.1)	4.5	5.9
Fiber Micronaire (mic units)[4]	4.6 (0.1)	4.6 (0.1)	4.2	5.0
Fiber Elongation (%)	6.0 (0.1)	6.2 (0.1)	5.6	8.1
Fiber Strength (g/tex)	31.2 (0.2)*	30.2 (0.2)	30.7	34.0
Fiber Uniformity (%)	83.5 (0.2)	83.4 (0.2)	82.8	84.3
Fiber Length (cm)	2.8 (0.0)	2.8 (0.0)	2.7	3.1

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and the conventional control (n = 44).
[1] Reference range = Minimum and maximum mean values across all 11 sites and nine references from the Study 2 field trial.
[2] SE = Standard error.
[3] DAP = days after planting.
[4] Measure of fiber fineness and maturity (expressed in dimensionless micronaire units).

Table G-14. Study 2 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Treated with Dicamba and Glufosinate Herbicides Compared to the Conventional Control

	Phenotypic Characteristic (units)									
	Stand count at 14 DAP[1] (# per plot)		Stand count at 30 DAP (# per plot)		Final Stand Count at Harvest		Plant Height at 30 DAP (cm)		Plant Height before harvest	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE)[2]	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	182.0 (4.4)	179.8 (2.3)	178.8 (4.8)	179.5 (2.9)	177.0 (5.0)	177.5 (3.5)	20.7 (0.7)	21.1 (0.5)	66.8 (2.0)	73.5 (5.1)
ARTI	169.3 (8.3)	164.8 (7.2)	167.5 (8.0)	164.3 (7.1)	166.5 (8.0)	163.8 (7.2)	33.2 (0.2)	32.8 (0.2)	122.2 (2.2)	126.9 (3.1)
GACH	128.0 (15.7)	132.8 (8.0)	126.0 (16.4)	127.8 (10.3)	123.5 (16.9)	129.5 (9.1)	23.9 (0.6)	25.8 (0.8)	103.5 (6.5)	110.2 (8.7)
GAJE	186.0 (2.3)	181.3 (2.7)	170.0 (2.1)	164.0 (3.0)	171.0 (2.8)	157.3 (8.4)	23.2 (1.0)*	26.0 (0.7)	81.7 (2.4)*	92.6 (7.9)
KSLA	170.0 (1.1)	162.5 (5.9)	167.5 (3.4)	158.5 (5.3)	162.0 (2.8)	150.0 (6.7)	16.6 (0.81)	18.3 (1.2)	113.6 (1.5)*	127.0 (3.3)
LACH	109.0 (6.4)	110.3 (5.4)	108.5 (5.7)	112.5 (7.6)	91.3 (2.7)	94.8 (2.9)	14.7 (0.4)	15.9 (0.8)	164.8 (3.6)*	180.0 (3.1)
NCBD	141.8 (2.5)	140.3 (3.2)	141.8 (2.2)	140.3 (3.6)	136.8 (3.6)	134.3 (2.3)	17.8 (0.5)	19.4 (0.7)	87.4 (3.6)	84.7 (4.8)
NCME	133.5 (8.3)	148.0 (19.5)	152.5 (3.4)	160.3 (9.7)	155.0 (7.0)	166.3 (8.3)	18.6 (0.6)	19.7 (0.9)	75.7 (2.0)*	85.0 (3.4)
NMLC	166.8 (4.6)	168.8 (11.9)	168.8 (3.4)	164.0 (2.4)	160.0 (3.1)	163.3 (5.8)	7.4 (0.1)	7.2 (0.2)	98.3 (2.2)	102.7 (1.9)
SCEK	183.5 (3.3)	191.5 (4.6)	182.8 (3.1)	190.3 (3.9)	180.3 (3.3)	188.8 (3.8)	15.6 (1.2)	17.9 (1.3)	107.0 (2.0)	111.7 (1.4)
TXPL	107.8 (19.8)	125.0 (10.4)	98.0 (19.1)	119.0 (8.8)	101.8 (20.0)	130.0 (11.3)	7.8 (0.4)	7.4 (0.5)	60.9 (1.8)	60.9 (1.3)

Table G-14. Study 2 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Treated with Dicamba and Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic Characteristic (units)							
	1st Vigor[3,4] rating		2nd Vigor[3,5] rating		Nodes above white flower (obs. 1)		Nodes above white flower (obs. 2)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Range	Range	Range	Range	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	3 – 5	3 - 4	4-5	3 - 5	5.6 (0.0)	5.7 (0.2)	3.7 (0.2)	3.6 (0.2)
ARTI	2 – 3	2 - 3	2-4	2 - 2	8.8 (0 .1)	8.5 (0.2)	6.6 (0.2)	6.5 (0.2)
GACH	4 – 5	2 - 5	4-6	4 - 6	5.8 (0.2)	5.3 (0.2)	4.7 (0.3)	4.1 (0.4)
GAJE	1 – 1	1 - 1	2-3	2 - 2	6.3 (0.4)	6.6 (0.5)	6.1 (0.2)*	5.7 (0.2)
KSLA	1 – 3	2 - 3	2-3	2 - 2	4.4 (0.3)	4.6 (0.2)	4.1 (0.4)	4.2 (0.6)
LACH	3 – 6	3 - 5	1-2	1 - 2	8.6 (0.2)*	7.6 (0.1)	8.2 (0.0)	8.4 (0.1)
NCBD	2 – 2	2 - 3	3-3	3 - 3	7.5 (0.2)	7.1 (0.5)	5.7 (0.3)*	3.9 (0.2)
NCME	3 – 5	1 - 5	2-3	2 - 3	4.9 (0.2)	5.2 (0.2)	3.6 (0.3)	3.3 (0.2)
NMLC	1 - 2	2 - 2	1-1	1 - 1	7.9 (0.1)	7.1 (0.2)	8.0 (0.2)	8.1 (0.3)
SCEK	3 - 4	3 - 6	2-3	3 - 3	7.7 (0.3)	7.4 (0.2)	5.1 (0.4)	4.5 (0.1)
TXPL	1 - 1	1 - 1	3-3	3 - 3	6.2 (0.3)*	5.7 (0.1)	5.7 (0.0)*	5.1 (0.1)

Table G-14. Study 2 - Individual Site Phenotypic Comparison – Growth and Development Characteristics - of MON 88701 Treated with Dicamba and Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic characteristics (units)			
	Nodes above white flower (obs. 3)		Seedcotton yield (kg/ha)	
	MON 88701	Control	MON 88701	Control
Site	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	2.3 (0.2)	2.4 (0.4)	2,705.9 (124.4)	2,301.7 (173.4)
ARTI	4.9 (0.0)*	4.7 (0.1)	4,109.3 (266.9)	3,869.0 (112.9)
GACH	4.0 (0.1)	3.6 (0.3)	4,107.9 (42.0)*	4,471.5 (99.5)
GAJE	3.8 (0.0)	3.8 (0.0)	1,977.6 (341.5)	1,548.4 (342.4)
KSLA	3.4 (0.1)*	2.5 (0.4)	4,182.0 (329.6)	3,726.5 (81.7)
LACH	5.7 (0.1)	5.7 (0.1)	1,940.4 (182.3)	2,046.9 (210.4)
NCBD	3.8 (0.3)*	3.0 (0.3)	4,025.4 (189.5)	3,792.7 (283.0)
NCME	2.1 (0.2)	1.9 (0.1)	1,785.4 (181.0)	1,610.6 (206.9)
NMLC	7.0 (0.7)	7.1 (0.2)	2,048.0 (74.4)*	1,479.3 (65.6)
SCEK	3.8 (0.3)	3.5 (0.1)	5,264.2 (106.1)	5,424.3 (148.4)
TXPL	3.7 (0.2)	3.5 (0.3)	4,307.3 (109.1)	4,534.5 (215.6)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and control (n = 44).
[1] DAP = Days after planting.
[2] SE = Standard error.
[3] Plant vigor was rated per plot using a rating scale of 1-9 where: 1-3 is excellent vigor, 4-6 is average vigor, and 7-9 is poor vigor.
[4] First plant vigor score range (minimum-maximum). The range of plant vigor score at 14 DAP for the references is as follows: ARPR 3-6; ARTI 2-3; GACH 4-6; GAJE 1-3; KSLA 2-5; LACH 4-7; NCBD 3-3; NCME 1-6; NMLC 2-5;SCEK 4-6; TXPL 1-1.
[5] Plant vigor score range (minimum-maximum). The range of plant vigor score at 30 DAP for the references is as follows: ARPR 4-6; ARTI 2-2; GACH 3-6; GAJE 2-2; KSLA 2-3; LACH 2-3; NCBD 3-3; NCME 2-3; NMLC 1-3; SCEK 2-3; TXPL 3-3.

Table G-15. Study 2 - Individual Site Phenotypic Comparison – Seed Characteristics - of MON 88701 Treated with Dicamba and Glufosinate Herbicides Compared to the Conventional Control

	Seed Characteristics							
	Seed index (g per 100 seed)		Total seed per boll (# per boll)		Mature seed per boll (# per boll)		Immature seed per boll (# per boll)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	9.1 (0.2)*	10.0 (0.4)	26.1 (0.8)*	23.9 (0.7)	23.2 (1.5)*	15.9 (0.6)	2.9 (0.9)*	8.0 (0.6)
ARTI	9.6 (0.1)*	12.0 (1.0)	27.7 (0.3)	26.8 (0.7)	22.5 (1.0)*	18.0 (0.6)	5.3 (1.0)*	8.9 (1.2)
GACH	9.0 (0.3)*	9.7 (0.3)	29.7 (0.7)*	27.4 (1.1)	27.2 (1.4)*	23.4 (1.1)	2.5 (0.7)	4.0 (0.6)
GAJE	8.1 (0.4)*	10.4 (0.5)	25.4 (1.8)	24.6 (0.7)	12.6 (0.7)	11.6 (0.1)	12.8 (1.7)	13.0 (0.8)
KSLA	12.3 (0.3)	12.8 (0.3)	25.8 (0.5)	24.9 (0.4)	23.7 (0.3)	22.7 (1.0)	2.1 (0.8)	2.2 (0.6)
LACH	9.5 (0.3)*	10.3 (0.2)	29.8 (1.0)	28.7 (1.2)	21.6 (2.2)	17.5 (1.9)	8.3 (1.8)	11.2 (0.8)
NCBD	9.2 (0.2)*	10.5 (0.2)	28.0 (0.7)	25.7 (1.3)	19.7 (0.6)	18.2 (0.9)	8.3 (1.2)	7.5 (0.5)
NCME	8.9 (0.2)*	10.2 (0.1)	29.0 (0.4)	27.6 (1.7)	23.1 (1.4)	23.0 (1.7)	5.9 (1.4)	4.6 (0.2)
NMLC	10.2 (0.1)	10.9 (0.2)	31.8 (0.8)	30.7 (1.1)	29.6 (1.4)	28.1 (1.4)	2.2 (0.7)	2.6 (0.4)
SCEK	8.5 (0.2)*	9.8 (0.2)	27.9 (1.3)	27.3 (1.3)	21.4 (1.5)	19.1 (1.4)	6.5 (1.9)	8.2 (1.2)
TXPL	9.9 (0.4)*	11.0 (0.3)	32.2 (0.9)	30.1 (0.9)	26.5 (0.8)	25.7 (0.7)	5.8 (1.1)	4.4 (1.1)

* Indicates a statistically significant difference between MON 88701 and control ($p \leq 0.05$).

Table G-16. Study 2 - Individual Site Phenotypic Comparison – Boll and Fiber Characteristics - of MON 88701 Treated with Dicamba and Glufosinate Herbicides Compared to the Conventional Control

	Fiber Boll weight (g/boll)		Fiber Micronaire (mic units)[1]		Fiber Elongation (%)		Fiber Strength (g/tex)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE[2])	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	4.1 (0.2)	4.1 (0.2)	5.0 (0.0)	4.9 (0.1)	5.3 (0.1)	5.7 (0.2)	30.3 (0.7)	30.5 (0.4)
ARTI	4.4 (0.1)	4.4 (0.1)	4.8 (0.0)	4.8 (0.1)	5.1 (0.1)	5.4 (0.2)	29.2 (0.4)	28.8 (0.4)
GACH	4.8 (0.1)	4.9 (0.1)	4.8 (0.1)	4.7 (0.1)	5.8 (0.3)	6.3 (0.3)	31.0 (0.4)*	29.5 (0.5)
GAJE	3.3 (0.2)	3.7 (0.1)	3.7 (0.2)	3.6 (0.1)	7.5 (0.1)	7.0 (0.3)	31.5 (0.2)	31.5 (0.5)
KSLA	5.4 (0.2)	5.3 (0.1)	4.4 (0.0)	4.4 (0.1)	6.1 (0.1)	6.2 (0.3)	30.7 (0.3)*	29.4 (0.3)
LACH	5.0 (0.2)	5.4 (0.3)	4.7 (0.2)	4.6 (0.1)	5.4 (0.1)	5.9 (0.3)	31.3 (0.3)*	29.9 (0.2)
NCBD	4.7 (0.1)	4.7 (0.2)	4.3 (0.1)	4.3 (0.1)	6.2 (0.2)	6.0 (0.2)	33.9 (0.2)	32.7 (0.4)
NCME	4.8 (0.1)	5.0 (0.3)	4.8 (0.1)	4.9 (0.1)	5.9 (0.2)	5.9 (0.4)	32.2 (0.7)	31.2 (0.3)
NMLC	5.3 (0.2)	5.1 (0.3)	4.9 (0.1)*	4.7 (0.1)	6.1 (0.2)	6.4 (0.2)	29.3 (0.3)*	27.3 (0.5)
SCEK	4.3 (0.2)	4.4 (0.4)	4.9 (0.0)	4.9 (0.0)	6.7 (0.2)	6.9 (0.2)	30.7 (0.3)*	30.0 (0.4)
TXPL	5.8 (0.1)	5.7 (0.1)	4.8 (0.1)	4.6 (0.1)	6.4 (0.3)	6.5 (0.2)	33.0 (0.4)*	31.0 (0.3)

Table G-16. Study 2 - Individual Site Phenotypic Comparison – Boll and Fiber Characteristics - of MON 88701 Treated with Dicamba and Glufosinate Herbicides Compared to the Conventional Control (continued)

	Fiber Uniformity (%)		Fiber Length (cm)	
	MON 88701	Control	MON 88701	Control
Site	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	83.9 (0.5)	84.6 (0.1)	2.8 (0.0)	2.9 (0.0)
ARTI	83.5 (0.2)	83.6 (0.1)	2.8 (0.0)	2.8 (0.0)
GACH	84.3 (0.6)	83.4 (0.4)	2.8 (0.1)	2.8 (0.0)
GAJE	82.6 (1.1)	82.5 (0.6)	2.8 (0.0)	2.8 (0.1)
KSLA	84.3 (0.3)*	83.3 (0.1)	3.1 (0.0)	3.0 (0.0)
LACH	83.7 (0.3)	83.4 (0.4)	2.8 (0.0)	2.8 (0.0)
NCBD	84.4 (0.4)	84.8 (0.6)	2.8 (0.0)	2.9 (0.0)
NCME	83.6 (0.2)	83.2 (0.6)	2.7 (0.0)	2.8 (0.1)
NMLC	81.4 (0.6)	81.1 (0.7)	2.7 (0.0)	2.7 (0.1)
SCEK	82.5 (0.5)*	83.9 (0.1)	2.7 (0.0)*	2.8 (0.0)
TXPL	84.2 (0.4)	83.9 (0.3)	2.8 (0.1)	2.8 (0.0)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and control (n = 4).
[1] Measure of fiber fineness and maturity (expressed in dimensionless micronaire units).
[2] SE = Standard error.

Table G-17. Study 2 - Individual Site Phenotypic Comparison – Plant Mapping - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

	Phenotypic Characteristic (units)									
	Total Mainstem Nodes (#)		Nodes to first fruiting branch (#)		Total bolls[1] (# per plant)		Total first-position bolls (# per plant)		Vegetative bolls (# per plant)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE)[2]	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	16.4 (0.4)	16.9 (0.5)	6.1 (0.1)	6.3 (0.2)	6.2 (0.4)	5.9 (0.6)	3.3 (0.1)	3.1 (0.3)	1.1 (0.3)	1.0 (0.3)
ARTI	20.9 (0.7)*	19.8 (0.2)	5.4 (0.1)	5.4 (0.2)	11.3 (1.2)	9.7 (0.9)	7.2 (1.0)	6.1 (0.4)	0.0 (0.0)	0.1 (0.1)
GACH	21.6 (0.6)	20.9 (0.4)	6.5 (0.5)*	7.9 (0.5)	9.9 (0.4)	9.4 (1.3)	4.1 (0.5)	3.2 (0.5)	2.4 (0.5)	3.5 (0.9)
GAJE	17.7 (0.4)	17.5 (0.7)	5.0 (0.2)*	5.8 (0.2)	9.0 (0.6)	10.1 (0.6)	5.8 (0.2)	5.9 (0.3)	0.2 (0.1)	0.3 (0.2)
KSLA	18.9 (0.4)	19.4 (0.6)	3.8 (0.1)	4.0 (0.3)	19.1 (0.2)*	20.6 (0.3)	4.7 (0.4)	5.2 (0.3)	13.5 (0.4)	13.9 (0.6)
LACH	20.9 (0.4)	22.8 (1.4)	7.1 (0.6)	6.9 (0.4)	7.1 (0.8)	4.9 (1.2)	2.9 (0.5)	2.8 (0.6)	1.8 (0.5)	0.4 (0.3)
NCBD	15.0 (0.4)	14.8 (0.6)	4.2 (0.1)	4.3 (0.2)	9.8 (0.4)	8.9 (0.5)	6.3 (0.5)	5.5 (0.3)	0.6 (0.2)	0.3 (0.1)
NCME	14.5 (0.5)	15.2 (0.2)	4.5 (0.1)	4.6 (0.1)	6.8 (0.7)*	4.2 (0.2)	3.5 (0.3)*	2.5 (0.1)	1.0 (0.2)*	0.1 (0.1)
NMLC	19.6 (0.2)	18.9 (0.3)	4.8 (0.5)	5.0 (0.4)	6.7 (0.7)*	4.5 (0.5)	4.4 (0.3)*	3.0 (0.4)	0.4 (0.1)	0.0 (0.0)
SCEK	15.8 (0.2)	15.8 (0.2)	4.6 (0.2)	4.6 (0.1)	9.7 (0.4)	10.0 (0.6)	6.9 (0.3)	6.3 (0.3)	0.3 (0.1)	0.7 (0.2)
TXPL	18.3 (0.2)	18.2 (0.3)	5.7 (0.1)	5.9 (0.2)	12.4 (0.5)	11.3 (0.4)	7.8 (0.2)*	7.1 (0.1)	1.0 (0.3)	0.8 (0.2)

Table G-17. Study 2 - Individual Site Phenotypic Comparison – Plant Mapping - of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic Characteristic (units)			
	% First-position boll retention		% First-position bolls over total bolls	
	MON 88701	Control	MON 88701	Control
Site	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	33.0 (1.9)	30.3 (3.7)	56.6 (3.6)	56.0 (4.4)
ARTI	45.4 (4.9)	42.3 (2.8)	63.9 (2.1)	66.8 (2.9)
GACH	26.8 (2.0)	23.0 (2.6)	42.7 (5.0)	36.6 (3.2)
GAJE	45.1 (1.5)	52.6 (4.9)	67.4 (2.3)*	61.8 (1.9)
KSLA	32.2 (2.0)	34.0 (2.4)	24.8 (1.9)	24.9 (1.3)
LACH	20.9 (2.8)	18.4 (4.8)	48.8 (1.0)	55.8 (1.9)
NCBD	58.8 (4.3)	52.5 (0.9)	66.5 (4.5)	64.5 (4.0)
NCME	35.6 (3.3)*	23.9 (0.8)	53.9 (2.7)	58.4 (1.7)
NMLC	30.1 (2.1)*	21.3 (3.3)	69.5 (3.0)	65.8 (2.8)
SCEK	62.2 (2.6)	57.0 (2.5)	73.2 (3.5)*	65.7 (1.9)
TXPL	72.5 (1.4)	69.6 (1.7)	65.8 (2.0)	64.9 (2.2)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and conventional control (n = 4).
[1] Total bolls = number of first-position bolls + number of second-position bolls + number of vegetative bolls.
[2] SE = Standard error.

Table G-18. Study 2 – Combined-Site Phenotypic Comparison – Plant Mapping - of MON 88701 Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Phenotypic Characteristic (units)	MON 88701 Mean (SE)[2]	Control Mean (SE)	Reference Range[1] Minimum	Reference Range[1] Maximum
Mainstem Nodes (# per plant)	18.3 (0.3)	18.2 (0.4)	16.0	21.6
Nodes to First Fruiting Branch (# per plant)	5.2 (0.1)	5.5 (1.1)	4.2	7.6
Total Bolls (# per plant)[3]	10.1 (0.6)	9.0 (0.7)	8.6	13.4
Total First-Position Bolls (# per plant)	5.2 (0.3)*	4.6 (0.3)	2.9	6.3
Total Vegetative Bolls (# per plant)	2.1 (0.6)	1.9 (0.6)	0.7	5.0
% Retention First-Position Bolls (per plant)	41.7 (2.3)	38.6 (2.6)	21.2	53.5
% First-Position Bolls of Total Bolls (per plant)	56.7 (2.1)	56.5 (2.1)	36.0	59.6

*Indicates a statistically significant difference ($\alpha = 0.05$).between the test and control (n = 44).
[1] Reference range = Minimum and maximum mean values among the commercial conventional reference varieties.
[2] SE = Standard error.
[3] Total Bolls = number of first-position bolls + number of second-position bolls + number of vegetative bolls.

Table G-19. Study 2 - Individual Site Phenotypic Comparison – Plant Mapping - of MON 88701 Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

	Phenotypic Characteristic (units)									
	Total Mainstem Nodes (#)		Nodes to first fruiting branch (#)		Total bolls[1] (# per plant)		Total first-position bolls (# per plant)		Vegetative bolls (# per plant)	
	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control	MON 88701	Control
Site	Mean (SE)[2]	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	16.3 (0.4)	16.9 (0.5)	6.0 (0.0)	6.3 (0.2)	5.7 (0.3)	5.9 (0.6)	3.4 (0.4)	3.1 (0.3)	0.7 (0.1)	1.0 (0.3)
ARTI	19.7 (0.1)	19.8 (0.2)	5.2 (0.1)	5.4 (0.2)	11.0 (0.3)	9.7 (0.9)	6.8 (0.3)	6.1 (0.4)	0.3 (0.1)	0.1 (0.1)
GACH	21.2 (0.9)	20.9 (0.4)	6.5 (0.5)*	7.9 (0.5)	8.7 (1.6)	9.4 (1.3)	3.8 (0.8)	3.2 (0.5)	2.0 (0.6)	3.5 (0.9)
GAJE	17.0 (0.2)	17.5 (0.7)	5.1 (0.1)*	5.8 (0.2)	9.5 (1.1)	10.1 (0.6)	5.5 (0.4)	5.9 (0.3)	0.7 (0.3)	0.3 (0.2)
KSLA	19.6 (0.7)	19.4 (0.6)	3.7 (0.1)	4.0 (0.3)	20.1 (0.7)	20.6 (0.3)	4.8 (0.3)	5.2 (0.3)	14.3 (0.7)	13.9 (0.6)
LACH	21.4 (0.8)	22.8 (1.4)	6.4 (0.4)	6.9 (0.4)	7.3 (1.1)	4.9 (1.2)	3.8 (0.5)	2.8 (0.6)	1.0 (0.4)	0.4 (0.3)
NCBD	16.5 (0.7)*	14.8 (0.6)	4.5 (0.2)	4.3 (0.2)	10.2 (1.0)	8.9 (0.5)	6.5 (0.4)*	5.5 (0.3)	0.4 (0.3)	0.3 (0.1)
NCME	15.1 (0.3)	15.2 (0.2)	4.8 (0.1)	4.6 (0.1)	7.1 (0.4)*	4.2 (0.2)	3.4 (0.3)*	2.5 (0.1)	1.1 (0.1)*	0.1 (0.1)
NMLC	19.4 (0.2)	18.9 (0.3)	4.8 (0.4)	5.0 (0.4)	7.7 (0.6)*	4.5 (0.5)	4.7 (0.2)*	3.0 (0.4)	0.3 (0.2)	0.0 (0.0)
SCEK	16.1 (0.4)	15.8 (0.2)	4.6 (0.1)	4.6 (0.1)	9.4 (0.3)	10.0 (0.6)	6.5 (0.3)	6.3 (0.3)	0.3 (0.0)*	0.7 (0.2)
TXPL	18.5 (0.7)	18.2 (0.3)	5.3 (0.2)	5.9 (0.2)	14.3 (1.8)*	11.3 (0.4)	8.1 (0.2)*	7.1 (0.1)	1.8 (0.9)	0.8 (0.2)

Table G-19. Study 2 - Individual Site Phenotypic Comparison – Plant Mapping - of MON 88701 Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

	Phenotypic Characteristic (units)			
	% Retention first-position bolls (per plant)		% First-position bolls of total bolls (per plant) [2]	
	MON 88701	Control	MON 88701	Control
Site	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
ARPR	33.5 (3.7)	30.3 (3.7)	61.4 (2.6)	56.0 (4.4)
ARTI	46.7 (2.0)	42.3 (2.8)	61.6 (1.5)	66.8 (2.9)
GACH	25.2 (4.2)	23.0 (2.6)	45.4 (3.7)*	36.6 (3.2)
GAJE	45.6 (2.8)	52.6 (4.9)	61.4 (2.0)	61.8 (1.9)
KSLA	32.0 (2.8)	34.0 (2.4)	24.5 (1.6)	24.9 (1.3)
LACH	25.5 (2.0)	18.4 (4.8)	57.5 (5.2)	55.8 (1.9)
NCBD	54.5 (1.4)	52.5 (0.9)	65.2 (3.1)	64.5 (4.0)
NCME	33.7 (2.4)*	23.9 (0.8)	50.5 (3.3)	58.4 (1.7)
NMLC	32.2 (1.5)*	21.3 (3.3)	64.8 (4.6)	65.8 (2.8)
SCEK	57.5 (3.0)	57.0 (2.5)	70.0 (2.7)	65.7 (1.9)
TXPL	72.1 (2.3)	69.6 (1.7)	61.0 (6.3)	64.9 (2.2)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and conventional control (n = 4).
[1] Total bolls = number of first-position bolls + number of second-position bolls + number of vegetative bolls.
[2] SE = Standard error.

Table G-20 Study 1 – Qualitative Assessment of Plant Response to Abiotic Stressors - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Abiotic Stressor	Number of observations across all sites	Number of observations where no differences were observed between MON88701 and the conventional control
Total	169	169
Compaction	4	4
Drought/ Dry	40	40
Flood	1	1
Hail	6	6
Heat	46	46
Nutrient deficiency	22	22
Wet soil/excess precipitation	17	17
Wind damage	33	33

Note 1: The experimental design was a randomized complete block with four replications. Data were not subjected to statistical analysis. No differences were observed between MON 88701 and the conventional control during any observation of plant response to abiotic stressors.

Table G-21. Study 1 – Qualitative Assessment of Disease Damage - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Disease	Number of observations across all sites	Number observations where no differences were observed between MON 88701 and the conventional control
Total	170	170
Anthracnose	2	2
Ascochyta leaf blight	2	2
Bacterial blight	23	23
Boll rot	26	26
Cotton leaf rust	13	13
Damping off	1	1
Fusarium wilt	14	14
Leaf spots[1]	43	43
Pythium	11	11
Reniform nematode	1	1
Rhizoctonia	16	16
Root-knot nematode	6	6
Thielaviopsis	1	1
Verticillium wilt	11	11

Note 1: The experimental design was a randomized complete block with four replications. Data were not subjected to statistical analysis. No differences were observed between MON 88701 and the conventional control during any observation of plant response to abiotic stressors.

[1] includes *Septoria*

Table G-22. Study 1 – Qualitative Assessment of Arthropod-related Damage - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Arthropod	Number of observations across all sites	Number observations where no differences were observed between MON 88701 and the conventional control
Total	159	159
Aphids (Aphididae)	31	31
Beet armyworms (*Spodoptera exigua)*	2	2
Cut worms (Noctuidae)	1	1
Fall armyworms (*Spodoptera frugiperda*)	4	4
Fleahoppers (*Pseudatomoscelis seriatus)*	4	4
Grasshoppers (Acrididae)	8	8
Heliothines (*Helicoverpa zea* and *Heliothis virescens*)	25	25
Southern corn rootworm beetles (*Diabrotica undecimpunctata howardi*)	3	3
Soybean loopers (*Pseudoplusia inclunes*)	2	2
Spider mites (*Tetranychus* spp.)	9	9
Stink bugs (Pentatomidae)	28	28
Tarnished plant bugs (*Lygus lineolaris*)	21	21
Thrips (Thripidae)	16	16
White flies (*Bemisia* spp.)	5	5

Note 1: The experimental design was a randomized complete block with four replications. Data were not subjected to statistical analysis. No differences were observed between MON 88701 and the conventional control during any observation of plant response to abiotic stressors.

Table G-23. Study 1 - Individual Site Analysis: Quantitative Assessment of Thrips Damage - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Rating[1]	Site	MON 88701 (SE)[2]	Control (SE)
1	ARAU	0.0 (0.0) †	0.0 (0.0)
	ARPR	0.9 (0.1)	0.8 (0.1)
	GACH	0.0 (0.0) †	0.0 (0.0)
	LABU	0.0 (0.0) †	0.0 (0.0)
	SCEK	1.5 (0.2)	1.4 (0.2)
2	ARAU	0.1 (0.0)	0.1 (0.0)
	ARPR	0.3 (0.1)	0.2 (0.1)
	GACH	0.0 (0.0) †	0.0 (0.0)
	LABU	0.0 (0.0) †	0.0 (0.0)
	SCEK	0.0 (0.0) †	0.0 (0.0)
3	ARAU	0.0 (0.0) †	0.0 (0.0)
	ARPR	0.1 (0.0)*	0.3 (0.1)
	GACH	0.0 (0.0) †	0.0 (0.0)
	LABU	0.0 (0.0) †	0.0 (0.0)
	SCEK	0.0 (0.0) †	0.0 (0.0)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and conventional control ($n = 4$).

† No p-values were generated due to lack of variability in the data.

[1] Thrips damage observation # 1 was made at approximately 14 DAP and the two subsequent observations at approximately seven day intervals thereafter.

[2] SE = Standard error.

Table G-24. Study 1 - Individual Site Analysis: Quantitative Assessment of Heliothine Damage - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

		Percent Damaged Fruiting Bodies		# of Live Larvae	
Observation[1]	Site	MON 88701 (SE)[2]	Control (SE)	MON 88701 (SE)	Control (SE)
1	ARAU	3.8 (1.0)	2.0 (0.7)	0.0 (0.0)	0.0 (0.0)
	ARPR	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)
	GACH	9.2 (2.0)	6.8 (2.5)	0.5 (0.2)*	0.1 (0.0)
	LABU	1.0 (0.6)	0.3 (0.3)	0.0 (0.0)	0.0 (0.0)
	SCEK	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
2	ARAU	0.2 (0.2)	0.6 (0.6)	0.0† (0.0)	0.0 (0.0)
	ARPR	1.6 (0.3)	2.2 (0.4)	0.1 (0.1)	0.2 (0.1)
	GACH	0.8 (0.5)	0.4 (0.4)	0.0 (0.0)	0.1 (0.1)
	LABU	2.9 (1.7)	2.6 (0.9)	0.1 (0.1)	0.0 (0.0)
	SCEK	21.2 (13.3)	26.0 (9.2)	0.6 (0.4)	0.3 (0.1)
3	ARAU	2.5 (1.0)	1.4 (0.6)	0.0 (0.0)	0.1 (0.1)
	ARPR	4.9 (0.6)	4.9 (1.4)	0.3 (0.1)	0.2 (0.1)
	GACH	4.1 (2.0)	3.3 (1.4)	0.1 (0.0)	0.1 (0.0)
	LABU	4.7 (1.1)	2.9 (0.9)	0.1 (0.1)	0.2 (0.1)
	SCEK	2.3 (2.3)	0.6 (0.6)	0.0 (0.0)	0.0 (0.0)
4	ARAU	8.7 (1.5)*	15.1 (2.4)	0.0 (0.0)	0.1 (0.1)
	ARPR	0.0† (0.0)	0.0 (0.0)	0.0† (0.0)	0.0 (0.0)
	GACH	1.5 (0.5)	2.8 (1.3)	0.0 (0.0)	0.0 (0.0)
	LABU	20.0 (3.8)	16.4 (2.4)	0.4 (0.1)	0.3 (0.1)
	SCEK	1.2 (0.5)	0.4 (0.4)	0.0 (0.0)	0.0 (0.0)

* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and the conventional control (n = 4).
[1] Heliothine damage observation 1 was made at approximately 45 DAP and the two subsequent observations at approximately 15 day intervals thereafter.
[2] SE = Standard Error.

Table G-25. Study 1 - Abundance of Pest Arthropods - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

		Pest Arthropod								
		Aphids (Aphididae)			Cabbage loopers (*Trichoplusia ni*)			Fall armyworms (*Spodoptera frugiperda*)		
Coll.[1]	Site	MON 88701 (SE)[2]	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Referencer ange	MON 88701 (SE)	Control (SE)	Referencer ange
1	ARAU	–	–	–	2.0 (0.9)	2.3 (0.5)	0.8 – 1.8	–	–	–
	ARPR	0.0 (0.0)	0.0 (0.0)	0.0 – 0.5	–	–	–	–	–	–
	GACH	3358.0 (656.5)	1971.8 (419.3)	1193.5 – 5796.0	–	–	–	–	–	–
	LABU	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	1.3 (0.6)	2.0 (0.4)	0.5 – 1.8	–	–	–
	SCEK	27.3 (8.2)	20.0 (6.4)	10.3 – 31.3	–	–	–	–	–	–
2	ARAU	36.8 (8.4)	30.0 (4.6)	30.0 – 144.3	–	–	–	–	–	–
	ARPR	24.3 (10.0)	17.3 (5.6)	16.0 – 30.3	1.3 (0.6)	1.0 (0.4)	0.5 – 1.5	–	–	–
	GACH	6.8 (1.6)	2.5 (0.9)	2.5 – 4.5	–	–	–	–	–	–
	LABU	54.3 (12.1)	51.0 (20.1)	70.0 – 156.0	–	–	–	–	–	–
	SCEK	4713.3 (1424.1)	7440.0 (1117.1)	994.0 – 6840.0	–	–	–	–	–	–
3	ARAU	6.0 (1.7)	10.3 (3.7)	8.0 – 23.0	5.3 (2.3)	3.0 (1.1)	1.8 – 6.0	–	–	–
	ARPR	15.8 (8.8)	19.0 (10.5)	12.3 – 15.3	0.0 (0.0)	0.3 (0.3)	0.0 – 0.0	–	–	–
	GACH	19.3 (6.5)	19.3 (6.6)	8.3 – 10.3	3.8 (1.6)	1.3 (1.0)	1.3 – 3.8	1.5 (1.5)	1.0 (0.6)	0.3 – 0.8
	LABU	4.8 (1.1)	10.0 (3.2)	3.8 – 18.0	0.3 (0.3)	0.3 (0.3)	0.0 – 0.8	–	–	–
	SCEK	6.3 (3.0)	8.5 (1.9)	2.0 – 10.5	0.8 (0.5)	2.0 (1.2)	0.5 – 2.5	–	–	–
4	ARAU	4.8 (1.3)	5.8 (1.5)	1.5 – 15.8	0.8 (0.3)	0.8 (0.5)	0.0 – 0.5	–	–	–
	ARPR	3.5 (0.9)	5.5 (1.2)	1.3 – 5.0	–	–	–	–	–	–
	GACH	2.0 (1.3)	4.3 (2.3)	0.8 – 4.5	–	–	–	–	–	–
	LABU	1959.3 (303.3)	1993.8 (492.9)	795.3 – 2218.5	–	–	–	–	–	–
	SCEK	130.0 (15.9)	145.5 (38.0)	70.0 – 183.8	–	–	–	–	–	–

Table G-25. Study 1 - Abundance of Pest Arthropods - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

Coll.	Site	Pest Arthropod								
		Fleahoppers (*Pseudatomoscelis seriatus)*			Heliothines (*Helicoverpa zea* and *Heliothis virescens*)			Southern Armyworms (*Spodoptera eridania*)		
		MON 88701 (SE)	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range
1	ARAU	–	–	–	–	–	–	–	–	–
	ARPR	–	–	–	–			–	–	–
	GACH	0.5 (0.3)	0.5 (0.5)	0.0 – 0.5	0.3 (0.3)	0.8 (0.5)	0.0 – 0.3	–	–	–
	LABU	–	–	–	–			0.3 (0.3)	0.5 (0.3)	0.8 – 2.0
	SCEK	–	–	–	–	–	–	–	–	–
2	ARAU	–	–	–	0.8 (0.5)	0.5 (0.3)	0.3 – 1.0	–	–	–
	ARPR	–	–	–	2.5 (1.0)	2.0 (0.9)	1.3 – 3.5	–	–	–
	GACH	–	–	–	0.3 (0.3)	0.3 (0.3)	0.0 – 1.3	–	–	–
	LABU	0.5 (0.5)	0.0 (0.0)	0.3 – 0.8	1.3 (0.6)	0.8 (0.5)	0.3 – 1.5	–	–	–
	SCEK	3.3 (1.0)	4.8 (3.0)	1.5 – 4.5	11.8 (4.6)	6.5 (2.7)	9.5 – 18.8	–	–	–
3	ARAU	–			0.8 (0.5)	2.0 (0.9)	0.3 – 2.3	–	–	–
	ARPR	–	–	–	0.3 (0.3)	0.8 (0.5)	0.3 – 2.5	–	–	–
	GACH	–		–	0.5 (0.5)	1.3 (1.3)	0.0 – 0.5	–	–	–
	LABU	–	–	–	1.5 (0.5)	0.8 (0.5)	0.5 – 1.8	–	–	–
	SCEK	6.0 (1.8)	6.0 (1.6)	2.8 – 9.5	1.3 (0.5)	0.8 (0.3)	0.5 – 1.5	–	–	–
4	ARAU	–	–	–	1.0 (0.7)	0.5 (0.3)	0.0 – 0.5	–	–	–
	ARPR	–	–	–	–	–	–	–	–	–
	GACH	0.3 (0.3)	0.0 (0.0)	0.0 – 0.0	–	–	–	–	–	–
	LABU	–	–	–	0.0 (0.0)	0.3 (0.3)	0.0 – 0.8	–	–	–
	SCEK	2.5 (0.9)	2.3 (1.0)	1.0 – 4.8	0.3 (0.3)	0.0 (0.0)	0.0 – 0.3	–	–	–

Table G-25. Study 1 - Abundance of Pest Arthropods - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

Coll.	Site	Pest Arthropod								
		Stink bugs (Pentatomidae)			Tarnished plant bugs (*Lygus lineolaris*)			Thrips (*Thripidae*)		
		MON 88701 (SE)	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range
1	ARAU	–	–	–	–	–	–	4.3 (1.7)	3.8 (2.3)	0.8 – 2.5
	ARPR	–	–	–	2.5 (0.9)	0.5 (0.3)	0.0 – 1.0	96.8 (31.9)	56.8 (17.6)	51.3 – 78.5
	GACH	–	–	–	–	–	–	5.0 (1.6)	3.5 (1.3)	2.3 – 4.8
	LABU	–	–	–	–	–	–	0.3 (0.3)	0.3 (0.3)	0.0 – 0.3
	SCEK	–	–	–	–	–	–	–	–	–
2	ARAU	0.8 (0.5)	0.3 (0.3)	0.0 – 1.5	1.0 (0.4)	0.8 (0.5)	0.0 – 1.5	–	–	–
	ARPR	–	–	–	1.8 (0.8)	3.3 (1.1)	2.0 – 4.0	24.0 (6.4)	25.3 (14.0)	17.5 – 32.5
	GACH	–	–	–	0.3 (0.3)	1.5 (0.7)	0.5 – 1.0	1.3 (0.5)	1.3 (0.3)	0.8 – 2.8
	LABU	–	–	–	1.3 (0.6)	0.8 (0.3)	0.5 – 0.8	1.0 (0.7)	1.5 (1.2)	0.5 – 1.5
	SCEK	–	–	–	–	–	–	13.8 (2.8)	16.5 (5.3)	15.0 – 30.5
3	ARAU	–	–	–	0.5 (0.5)	1.8 (0.9)	0.3 – 0.5	–	–	–
	ARPR	–	–	–	0.0 (0.0)	0.3 (0.3)	0.3 – 2.0	2.8 (2.1)	1.0 (0.7)	1.3 – 2.3
	GACH	–	–	–	–	–	–	24.0 (8.8)	19.5 (5.6)	10.5 – 25.0
	LABU	–	–	–	–	–	–	1.0 (0.4)	0.8 (0.3)	0.3 – 2.8
	SCEK	–	–	–	–	–	–	3.5 (1.8)	2.5 (0.9)	2.3 – 5.3
4	ARAU	1.8 (0.9)	1.3 (0.6)	0.3 – 1.5	–	–	–	2.0 (1.4)	0.8 (0.3)	0.8 – 1.8
	ARPR	0.5 (0.3)	0.3 (0.3)	0.5 – 1.3	1.3 (1.3)	0.3 (0.3)	0.8 – 1.5	8.3 (1.7)	9.0 (2.7)	9.3 – 18.5
	GACH	–	–	–	–	–	–	1.3 (1.0)	0.5 (0.3)	0.0 – 0.8
	LABU	0.3 (0.3)*	1.8 (0.5)	0.5 – 1.0	0.5 (0.5)*	2.0 (0.6)	0.5 – 2.0	1.5 (1.2)	1.3 (0.8)	1.5 – 10.0
	SCEK	–	–	–	–	–	–	11.0 (3.5)	6.0 (1.8)	11.3 – 20.0

Table G-25 Study 1 - Abundance of Pest Arthropods - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

Coll.	Site	White flies (*Bemisia* spp.) MON 88701 (SE)	Control (SE)	Reference range
1	ARAU	–	–	–
	ARPR	0.0 (0.0)	0.3 (0.3)	0.0 – 0.5
	GACH	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
	LABU	–	–	–
	SCEK	–	–	–
2	ARAU	0.5 (0.3)	0.3 (0.3)	0.5 – 1.5
	ARPR	–	–	–
	GACH	1.3 (0.3)	0.5 (0.3)	1.3 – 2.3
	LABU	–	–	–
	SCEK	0.5 (0.5)	0.5 (0.5)	0.0 – 0.5
3	ARAU	0.0 (0.0)	0.3 (0.3)	0.0 – 0.5
	ARPR	–	–	–
	GACH	–	–	–
	LABU	–	–	–
	SCEK	–	–	–
4	ARAU	–	–	–
	ARPR	2.0 (0.7)	3.0 (1.4)	0.8 – 3.0
	GACH	1.5 (0.7)	0.3 (0.3)	0.3 – 1.0
	LABU	–	–	–
	SCEK	1.3 (1.0)	0.5 (0.3)	0.0 – 0.8

Note: A dash (-) indicates arthropod not evaluated.
* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and the conventional control (n = 4).
[1] Arthropod collection 1 was made at approximately 30 DAP and the three subsequent collections at approximately 30 day intervals thereafter
[2] SE = Standard error.

Table G-26. Study 1 - Abundance of Beneficial Arthropods - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

		Beneficial Arthropod								
		Big eyed bugs (*Geocoris* spp.)			Braconids (Braconidae)			Damsel bugs (*Nabis* spp.)		
Coll.[1]	Site	MON 88701 (SE)[2]	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range
	ARAU	–	–	–	–	–	–	1.3 (0.5)	1.0 (0.7)	0.0 – 1.8
	ARPR	2.3 (0.6)	0.5 (0.5)	0.8 – 2.3	–	–	–	0.5 (0.5)	1.3 (0.6)	0.3 – 0.8
1	GACH	–	–	–	–	–	–	0.8 (0.5)	0.5 (0.3)	0.0 – 0.5
	LABU	–	–	–	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	–	–	–
	SCEK	0.3 (0.3)	0.0 (0.0)	0.3 – 1.0	–	–	–	–	–	–
	ARAU	0.3 (0.3)	0.0 (0.0)	0.0 – 1.3	–	–	–	–	–	–
	ARPR	1.0 (0.4)	1.5 (0.5)	0.3 – 0.8	–	–	–	–	–	–
2	GACH	10.5 (1.3)	11.8 (1.9)	6.8 – 16.5	–	–	–	6.0 (2.0)*	2.3 (0.8)	2.5– 7.3
	LABU	1.5 (0.7)	2.3 (1.3)	0.5 – 3.3	–	–	–	0.5 (0.3)	0.0 (0.0)	0.0 – 0.5
	SCEK	0.8 (0.5)	1.3 (0.5)	1.0 – 3.3	–	–	–	–	–	–
	ARAU	0.0 (0.0)	0.3 (0.3)	0.0 – 1.3	–	–	–	–	–	–
	ARPR	–	–	–	–	–	–	–	–	–
3	GACH	1.0 (0.4)	2.5 (0.9)	1.5 – 4.5	–	–	–	3.3 (1.0)	1.5 (0.9)	1.5 – 3.0
	LABU	–	–	–	–	–	–	–	–	–
	SCEK	7.0 (0.7)	6.0 (2.0)	5.0 – 13.0	–	–	–	–	–	–
	ARAU	1.5 (0.5)	2.5 (0.7)	1.5 – 3.5	–	–	–	1.8 (0.5)	3.0 (0.7)	0.8 – 2.5
	ARPR	7.5 (3.0)	4.0 (0.7)	3.0 – 7.5	–	–	–	–	–	–
4	GACH	5.0 (1.6)	5.5 (3.0)	1.3 – 8.5	–	–	–	3.3 (0.8)	4.8 (1.9)	1.3 – 4.0
	LABU	–	–	–	0.5 (0.5)	2.0 (1.4)	3.0 – 8.8	–	–	–
	SCEK	27.8 (9.4)	35.0 (4.8)	16.5 – 44.3	–	–	–	–	–	–

Table G-26. Study 1 - Abundance of Beneficial Arthropods - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

Coll.	Site	Beneficial Arthropod								
		Lacewings (*Chrysopa* spp.and *Hemerobius* spp.)			Ladybird Beetles (Coccinellidae)			*Orius* spp.		
		MON 88701 (SE)	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range	MON 88701 (SE)	Control (SE)	Reference range
	ARAU	–	–	–	0.5 (0.3)	0.5 (0.3)	0.3 – 0.8	0.0 (0.0)	0.3 (0.3)	0.0 – 0.3
	ARPR	–	–	–	2.3 (0.5)	3.3 (1.1)	1.0 – 2.5	1.8 (1.1)	0.5 (0.3)	0.0 – 2.8
1	GACH	0.8 (0.8)	0.3 (0.3)	0.3 – 0.5	14.3 (4.6)	12.3 (3.9)	6.0 – 43.8	0.8 (0.5)	0.0 (0.0)	0.3 – 0.8
	LABU	–	–	–	0.5 (0.5)	0.3 (0.3)	0.0 – 0.8	–	–	–
	SCEK	–	–	–	0.5 (0.3)	0.8 (0.3)	0.3 – 1.3	–	–	–
	ARAU	3.0 (0.4)	3.5 (0.9)	2.3 – 5.0	1.0 (0.4)	1.0 (0.7)	1.0 – 2.0	0.0 (0.0)*	1.5 (0.7)	0.3 – 0.8
	ARPR	2.5 (0.7)	3.0 (1.3)	1.8 – 4.0	9.0 (2.5)	4.8 (0.3)	5.5 – 8.5	1.0 (0.6)	1.0 (0.4)	0.5 – 1.8
2	GACH	0.3 (0.3)	1.0 (0.4)	1.0 – 2.0	10.8 (2.3)	10.0 (1.4)	3.3 – 12.3	–	–	–
	LABU	–	–	–	8.5 (1.4)	9.5 (2.4)	8.0 – 12.5	0.3 (0.3)	0.0 (0.0)	0.0 – 0.5
	SCEK	3.3 (0.8)	2.5 (1.0)	1.8 – 3.5	34.8 (6.5)	46.3 (10.8)	18.3 – 36.3	8.5 (3.1)	6.3 (1.9)	5.3 – 9.5
	ARAU	2.8 (1.3)	4.0 (0.7)	1.8 – 3.5	7.3 (3.2)	10.3 (2.7)	9.3 – 18.3	0.5 (0.3)*	2.8 (1.2)	0.8 – 3.3
	ARPR	1.0 (0.4)	1.5 (0.9)	0.5 – 1.5	6.5 (1.0)	6.0 (1.8)	5.5 – 8.0	0.3 (0.3)	1.0 (0.6)	0.0 – 0.5
3	GACH	3.0 (1.8)	4.0 (1.2)	2.0 – 5.5	12.0 (3.2)	11.0 (2.4)	4.8 – 10.3	–	–	–
	LABU	–	–	–	–	–	–	–	–	–
	SCEK	0.3 (0.3)	1.0 (0.4)	0.3 – 0.8	7.8 (0.9)	5.0 (0.7)	6.3 – 8.3	2.0 (1.7)	3.5 (0.9)	1.5 – 5.0
	ARAU	4.3 (1.6)	2.5 (0.5)	1.8 – 3.5	10.8 (2.5)	12.0 (1.7)	9.3 – 13.0	–	–	–
	ARPR	3.3 (1.1)	3.8 (1.3)	0.5 – 4.3	6.5 (1.6)	5.3 (1.0)	2.0 – 4.0	0.0 (0.0)	0.0 (0.0)	0.0 – 0.8
4	GACH	0.0 (0.0)	0.0 (0.0)	0.3 – 0.5	1.3 (0.6)	2.0 (1.1)	3.3 – 7.0	–	–	–
	LABU	0.3 (0.3)	0.3 (0.3)	0.5 – 2.3	6.3 (3.3)	3.5 (1.3)	4.0 – 8.0	3.8 (3.1)	3.3 (2.0)	2.8 – 10.3
	SCEK	1.0 (0.4)	0.3 (0.3)	0.5 – 1.8	7.5 (0.3)	5.0 (2.4)	5.0 – 10.8	3.8 (0.5)	4.0 (0.8)	3.0 – 10.3

Table G-26. Study 1 - Abundance of Beneficial Arthropods - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control (continued)

Coll.	Site	Spiders (Araneae) MON 88701 (SE)	Control (SE)	Reference range
1	ARAU	1.0 (0.4)	1.0 (0.4)	0.3 – 1.3
	ARPR	1.5 (0.5)	1.0 (0.7)	0.8 – 1.8
	GACH	1.5 (0.9)	2.5 (0.7)	0.3 – 2.0
	LABU	0.3 (0.3)	1.0 (1.0)	0.3 – 1.3
	SCEK	0.5 (0.5)	0.8 (0.3)	0.5 – 1.0
2	ARAU	1.0 (0.4)	1.3 (0.5)	0.3 – 2.0
	ARPR	6.0 (1.3)	4.0 (0.4)	2.5 – 5.8
	GACH	4.5 (0.3)	6.0 (1.2)	4.3 – 6.8
	LABU	5.3 (1.5)	3.0 (1.3)	2.8 – 5.3
	SCEK	2.8 (0.5)	4.3 (1.3)	1.8 – 4.3
3	ARAU	2.0 (0.4)	2.0 (1.1)	0.8 – 1.8
	ARPR	0.3 (0.3)	0.0 (0.0)	0.0 – 0.5
	GACH	4.0 (0.4)	2.8 (0.5)	3.8 – 4.3
	LABU	–	–	–
	SCEK	4.3 (1.0)	2.8 (0.9)	3.5 – 7.8
4	ARAU	3.3 (1.0)	3.5 (0.9)	3.5 – 7.3
	ARPR	1.5 (0.3)	2.0 (1.0)	0.3 – 1.8
	GACH	3.0 (0.8)	5.8 (1.8)	3.5 – 6.0
	LABU	0.8 (0.5)	0.8 (0.5)	0.3 – 1.5
	SCEK	6.5 (1.3)	6.0 (0.8)	8.0 – 12.5

Note: A dash (-) indicates arthropod not evaluated.
* Indicates a statistically significant difference ($\alpha = 0.05$) between MON 88701 and the conventional control (n = 4).
[1] Arthropod collection 1 was made at approximately 30 DAP and the three subsequent collections at approximately 30 day intervals thereafter
[2] SE = Standard error.

Table G-27 Study 2 – Qualitative Assessment of Plant Response to Abiotic Stressors - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Abiotic Stressor	Number of observations across all sites	Number of observations where no differences were observed between MON 88701 and the control
Total	127	127
Compaction	4	4
Drought/ Dry	30	30
Flood	1	1
Hail Damage	6	6
Heat	30	30
Nutrient deficiency	10	10
Wet soil/excess precipitation	17	17
Wind damage	29	29

Note 1: The experimental design was a randomized complete block with four replications. Data were not subjected to statistical analysis. No differences were observed between MON 88701 and the conventional control during any observation of plant response to abiotic stressors.

Table G-28. Study 2 – Qualitative Assessment of Disease Damage of MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Disease	Number of observations across all sites	Number of observations where no differences were observed between MON 88701 and the control
Total	129	129
Anthracnose	3	3
Ascochyta leaf blight	3	3
Bacterial blight	14	14
Boll rot	15	15
Cotton leaf rust	7	7
Damping off	1	1
Fusarium wilt	11	11
Leaf spots[1]	36	36
Pythium	9	9
Reniform nematode	1	1
Rhizoctonia	12	12
Root-knot nematode	6	6
Thielaviopsis	1	1
Verticillium wilt	10	10

Note 1: The experimental design was a randomized complete block with four replications. Data were not subjected to statistical analysis. No differences were observed between MON 88701 and the conventional control during any observation of plant response to abiotic stressors

[1] Includes *Alternaria* and *Septoria*

Table G-29. Study 2 – Qualitative Assessment of Arthropod-related Damage - MON 88701 Not Treated with Dicamba or Glufosinate Herbicides Compared to the Conventional Control

Arthropod	Number of observations across all sites	Number of observations where no differences were observed between MON 88701 and the control
Total	129	129
Aphids (Aphididae)	24	24
Beet armyworms (*Spodoptera exigua)*	1	1
Cabbage loopers (*Trichoplusia ni*)	1	1
Cut worms (Noctuidae)	3	3
Fall armyworms (*Spodoptera frugiperda*)	4	4
Fleahoppers (*Pseudatomoscelis seriatus)*	2	2
Grasshoppers (Acrididae)	6	6
Heliothines (*Helicoverpa zea* and *Heliothis virescens*)	23	23
Southern corn rootworm beetles (*Diabrotica undecimpunctata howardi*)	2	2
Soybean loopers (*Pseudoplusia inclunes*)	1	1
Spider mites (*Tetranychus* spp.)	9	9
Stink bugs (Pentatomidae)	21	21
Tarnished plant bugs (*Lygus lineolaris*)	14	14
Thrips (Thripidae)	16	17
White flies (*Bemisia* spp.)	2	2

Note 1: The experimental design was a randomized complete block with four replications. Data were not subjected to statistical analysis. No differences were observed between MON 88701 and the conventional control during any observation of plant response to abiotic stressors.

References for Appendix G

Drees, B.M. and M.E. Rice. 1985. The vertical beat sheet: A new device for sampling soybeans insects. Journal of Economic Entomology 78:1507-1510.

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

Appendix H: Materials and Methods for Pollen Morphology and Viability Assessment

H.1. Plant Production

MON 88701, the conventional control, and four commercial reference varieties were grown under similar agronomic conditions in a field trial in Crittenden County, Arkansas (Table G-1; ARPR site). The trial was arranged in a randomized complete-block design with four replications. Each plot consisted of eight rows approximately 6 m in length.

H.2. Flower Collection and Sample Preparation

Five flowers, each open less than 24 hours at the time of collection, were collected from each plot. The pollen obtained from an individual flower comprised a subsample of the plot and was placed in a uniquely labeled, clean container. Six hundred µl of Alexander's stain (Alexander, 1980) diluted 1:5 with distilled water was added to each container, and the container contents were thoroughly mixed. Containers were placed on wet (water) ice within 10 minutes of pollen collection. After transport to the performing laboratory, the pollen in the containers was allowed to stain at ambient temperatures for at least 20 hours.

H.3. Data Collection

Pollen subsamples were assessed for pollen viability, diameter, and general morphology. Slides were prepared by aliquoting 30 µl of suspended pollen/stain solution onto a slide. The slides were viewed under an Olympus BX51TRF light/fluorescence microscope with an Olympus DP70 digital color camera. The associated PC computer had imaging software for diameter measurement (I-Pro Plus version 6.2.1.491© 1993-2007, Media Cybernetics, Inc.) and camera software (DP Controller 1. 2. 1.108 © 2001-2003, Olympus Optical Co., Ltd. and DP Manager version 1, 2, 1, 107 © 2001-2003, Olympus Optical Co., Ltd.).

H.3.1. Pollen Viability

To assess pollen viability, 77 or more pollen grains were evaluated under the 40X ocular lens (400X total magnification) for each subsample. When exposed to the staining solution, viable pollen grains stained purple because of the presence of vital cytoplasmic content, while dead pollen grains stained clear to light blue-green. In addition, viable pollen grains appeared round, whereas non-viable pollen grains appeared round to collapsed depending on the degree of hydration.

H.3.2. Pollen Diameter

Pollen diameter was measured under the 40X ocular lens (400X total magnification) using software (Image-Pro Plus version 6.2.1.491© 1993-2007 Media Cybernetics, Inc.)

to view digital images. For each replication, pollen diameter was measured along two perpendicular axes for ten representative viable pollen grains.

H.3.3. General Pollen Morphology

General morphology of the pollen was observed for each subsample of MON 88701, the conventional control, and the commercial reference varieties during determination of pollen viability.

H.4. Statistical Analysis

Monsanto Statistics Technology Center performed the statistical analysis. An analysis of variance was conducted according to a randomized complete block design using SAS® Version 9.2 (SAS, 2008) with a significance level of 5% ($p \leq 0.05$). MON 88701 was compared to the conventional control for percent viable pollen and pollen grain diameter. MON 88701 was not statistically compared to the reference varieties. A reference range for each measured characteristic was determined from the minimum and maximum mean values from among the four commercial reference varieties. General pollen morphology was qualitative; therefore, no statistical analysis was conducted on these observations.

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Table H-1. Starting Seed for Pollen Morphology and Viability Assessment

Material	Phenotype	Monsanto ID
Coker 130	Conventional	11268128
Nex Gen NG3410RF	Glyphosate-tolerant	11266969
ST474	Conventional	11266156
DP 493	Conventional	11266763
SG125	Conventional	11266155
MON 88701	Dicamba and glufosinate-tolerant	11268129

References for Appendix H

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

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

Appendix I: Herbicide Resistance

I.1. Introduction

Based upon theory of natural selection, plant populations can develop resistance to an herbicide due to the selection of individuals that carry specific genes that can render those individuals unaffected by the typical lethal effects of an herbicide. The application of an herbicide to the plant does not, itself, cause a mutation in subsequent generations. Rather, over time, those few plant biotypes containing resistant gene(s) become dominant in the population with repeated use of the herbicide in the absence of other control methods, such as use of other herbicides and/or use of cultural control methods. The development of resistant populations is a possibility for all herbicides. The probability for resistance to develop is a function of: frequency of resistant allele(s)[8], mechanism of resistance, dominance or recessive nature of the resistant allele(s), relative fitness of the resistant biotype, and frequency or duration of herbicide use in the absence of other control methods (Beckie, 2006; Jasieniuk, et al., 1996; Sammons et al., 2007). The probability of resistance is not the same for all herbicides, with some herbicides (*e.g.*, ALS and ACCase classes) exhibiting resistance more quickly than other herbicides (*e.g.* auxin class, glyphosate, dinitroanilines class).

Herbicide resistance can become a limiting factor in crop production if the resistant weed population cannot be controlled with other herbicides or cultural practices. In general, this has not been the case for any herbicide. In most crops, there are multiple herbicide options for growers to use. However, good management practices to delay the development of herbicide resistance have been identified and are being actively promoted by the public and private sectors (HRAC, 2010) and are being implemented by growers.

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. Stewardship of the dicamba and glufosinate herbicides to preserve their usefulness for growers is an important aspect of Monsanto's stewardship commitment. Although herbicide resistance may eventually occur in weed species when any herbicide is widely used, resistance can be postponed, contained, and managed through research, education, and good management practices. These are the key elements of Monsanto's approach to providing stewardship of dicamba and glufosinate used on MON 88701 integrated into the glyphosate-tolerant cotton systems. Monsanto will invest in research, and grower/retailer education and training programs to provide information on best practices to manage dicamba and glufosinate weed resistance in cotton production. This appendix provides an overview of Monsanto's approach to the development of best management practices to mitigate dicamba and glufosinate weed resistance. Monsanto works closely with weed scientists in academia and with other companies to research and develop best management practices and to uniformly communicate such practices to growers. Evidence of this cooperative effort is the recent development and posting of herbicide-resistant training modules on the WSSA website

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[8] An allele is any of several forms of a gene, usually arising through mutation, that are responsible for hereditary variation.

(www.wssa.net) and the publication of guidelines by the Herbicide Resistance Action Committee (HRAC) on their website (www.hracglobal.com).

I.2. The Herbicide Dicamba

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is classified as a benzoic acid herbicide belonging to the synthetic auxin group of herbicides (HRAC, 2010). The herbicides in this group act as growth regulators similar to endogenous indole acetic acid (IAA) but are structurally diverse. The synthetic auxin group includes five chemical families (benzoic acid, pyridine-carboxylic acid, quinoline carboxylic acid, phenoxy-carboxylic acid and a separate class which includes one herbicide, benazolin ethyl). The specific site of action among the different synthetic auxin chemical families may be different. In addition to dicamba, specific herbicides in this group include 2,4-D, 2,4-DB, mecoprop, MCPA, clopyralid, picloram, quinclorac and several other active ingredients. Dicamba and other synthetic auxin herbicides are classified in Herbicide Group 4 by the Weed Science Society of America (HRAC, 2009). Most herbicides in this group are active on broadleaf weeds only, but a few have significant activity on grasses, *e.g.*, quinclorac. Dicamba provides preemergence and postemergence control of over 95 annual and biennial broadleaf weed species and control or suppression of over 100 perennial broadleaf and woody species (BASF, 2008). Dicamba is not active on grass weeds and is often used in combination with other herbicides to provide broad spectrum weed control.

Dicamba herbicide was commercialized in the U.S. for agricultural use in 1967 and is currently labeled for preemergence and/or postemergence weed control in corn, soybean, cotton, sorghum, small grains (wheat, barley and oats), millet, pasture, rangeland, asparagus, sugarcane, turf, grass grown for seed, conservation reserve program land, and fallow cropland, and for non-crop uses (U.S. EPA, 2009). Dicamba is sold as standalone formulation which can be tank-mixed with one or more active ingredients depending upon the crop and the weed spectrum. Dicamba is also sold as a premix formulation with other herbicides.

Dicamba acts in plants by mimicking naturally-occurring plant growth hormones called auxins, thereby destroying tissue through uncontrolled cell division and growth (Ahrens, 1994). Ahrens (1994) further states that dicamba has been found to affect cell wall integrity and nucleic acid metabolism whereas in other cases it has been found to increase cell wall permeability, leading to cell enlargement. At low concentrations, dicamba has been found to increase synthesis of DNA, RNA, and proteins, resulting in altered cell division and growth. At high concentrations, inhibition of cell division and growth occur. In general, dicamba and other synthetic auxin herbicides have been found to affect multiple plant physiological systems. Grossmann (2010), in a review of auxin herbicides, outlined a proposed mechanism and mode-of-action for auxin herbicides and IAA at supraoptimal endogenous concentrations in dicot plant species. The proposal was based upon recent identification of receptors for auxins and hormone interaction in signaling between auxin, ethylene, and the upregulations of abscisic acid biosynthesis which would account for a large part of the various auxin-herbicide-mediated responses that are seen in sensitive dicots. In addition, research has indicated that there is a high

level of redundancy in auxin receptors which may account for the lack of development of widespread resistance to this herbicide group (Walsh et al., 2006).

Dicamba is taken up by plants through the roots, stems, and foliage (Ahrens, 1994; NPIC, 2002). Dicamba translocates to all plant tissues but accumulates in growing tissues. Translocation of dicamba is typically slower in tolerant plants such as grasses compared to broadleaf plants.

I.3. The Herbicide Glufosinate

Glufosinate [2-Amino-4-(hydroxymethylphosphinyl) butanoic acid] is classified as a phosphinic acid herbicide belonging to the glutamine synthetase inhibitor group of herbicides (HRAC, 2010). Bialaphos is the only other active ingredient belonging to the phosphinic acid chemical family. Glufosinate and bialaphos are classified in Herbicide Group 10 by the Weed Science Society of America (HRAC, 2010). Glufosinate provides postemergence control of over 90 annual grass and broadleaf weed species and 25 biennial and perennial grass and broadleaf weed species.

Glufosinate was first approved for use in the U.S. in 1994 (U.S. EPA, 2008) and is currently labeled for non-crop uses, preplant burndown to glufosinate-tolerant and non-tolerant crops and/or in-crop postemergence weed control in glufosinate-tolerant canola, corn, cotton, and soybean, (Bayer CropScience, 2011). Glufosinate is sold as standalone formulation which can be tank mixed with one or more active ingredients depending upon the crop and the weed spectrum.

Glufosinate acts in plants by inhibiting the enzyme glutamine synthase, causing a toxic buildup of ammonia within the treated plant (Bayer, 2010). Glufosinate is a nonselective herbicide and has no residual activity. This herbicide has a different mode-of-action than the other major herbicides used in cotton.

I.4. Herbicide-Resistant Weeds and Resistance Management Strategies

The development of herbicide-resistant weeds is not a new phenomenon and resistance is not limited to certain select herbicides. In 1957, the first U.S. herbicide-resistant weed, a spreading dayflower biotype resistant to 2,4-D, was identified in Hawaii (Heap, 2012a). See Table VIII-4 for scientific names of weeds mentioned in Appendix I. Through November 2011, there are approximately 80 individual weed species with known herbicide-resistant biotypes to one or more herbicides in the U.S. For example, there are 45 weed species resistant to ALS herbicides, 16 to ACCase inhibitors, 24 to photosystem II inhibitors, and 13 to glycine herbicides (Heap, 2012b). Growers have been managing herbicide-resistant weeds for decades with the use of alternative herbicides and/or cultural methods such as tillage or crop rotation.

The occurrence of an herbicide-resistant weed biotype does not end the useful lifespan or preclude the effective use of the herbicide as part of an overall diversified weed management system. The three herbicide classes with the highest number of resistant species, ALS, ACCase and triazine herbicides, are still effectively used by growers today.

It is important to distinguish herbicide resistance from herbicide tolerance. A herbicide resistant weed is one in which there is an inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA, 2012). A herbicide-tolerant weed species is one that is naturally tolerant to a herbicide, for example a grass species is not killed by the application of a broadleaf herbicide (WSSA, 2012). Furthermore, certain weed species, while neither resistant nor tolerant, are inherently difficult to control with a particular herbicide, requiring more careful herbicide use and weed management practices.

Since the first confirmed cases of herbicide resistance, research has been directed at determining which practices are best for managing existing resistance situations and how best to reduce the development of herbicide resistance. Resistance management practices most often recommended by University/Cooperative Extension Service (CES) and industry are: 1) use of multiple herbicide modes-of-action in mixture, sequence, or in rotation; 2) crop rotation; 3) use of cultural control measures such as tillage and time of planting; and 4) use of the labeled herbicide rate at the recommended timing of application (Gressel and Segel, 1990; Beckie, 2006). Recent research by Beckie and Reboud (2009) indicates that in some cases herbicide mixtures offer a better management option than rotating herbicides. Simultaneously using two herbicides with different modes-of-action, each effective on the same weed species, significantly reduce the probability of weeds developing resistance to either or both herbicides (Beckie and Reboud, 2009). Crop rotation is also an effective method for resistance management due to the fact that it fosters the use of additional herbicide modes-of-action and, potentially, use of additional cultural practices to manage weeds over time. The use of multiple methods of weed control in a single location is the technical basis for management programs to delay the development of resistance. This general concept has been referred to as applying "diversity" within a crop or across a crop rotation (Beckie, 2006; Powles, 2008).

It is generally accepted that conservation tillage practices (minimum-till and no-till) create environments where herbicide resistance is more likely to develop (Beckie, 2006). This is primarily due to selection pressure put on weeds by herbicide use due to the absence of tillage as a cultural weed management practice to supplement herbicide use. However, this is not always the case. Legere et al. (2000) found that an increase in the use of ACCase inhibitors in a conservation tillage system (*e.g.*, aryloxyphenoxy propionates and phenylpyrazolines herbicide families) did not result in an increased incidence of wild oat populations resistant to ACCase inhibitors. In conclusion, conservation tillage practices should not be considered a primary contributing factor to the development of resistance in all cases.

I.5. Characteristics of Herbicides and Herbicide Use Influencing Resistance

While the incidence of weed resistance is often associated with repeated applications of an herbicide, the actual probability for the development of resistant populations is related, in part, to the specific herbicide active ingredient, chemical family and the herbicide group. Some herbicides are more prone to the development of resistance than others (Heap, 2012c). The graph in Figure I-1 illustrates the global instances of weed resistance

to various herbicide groups. The different slopes of observed resistance are largely due to the factors described above, which relate to the specific herbicide active ingredient as well as to the group and herbicide family and its function.

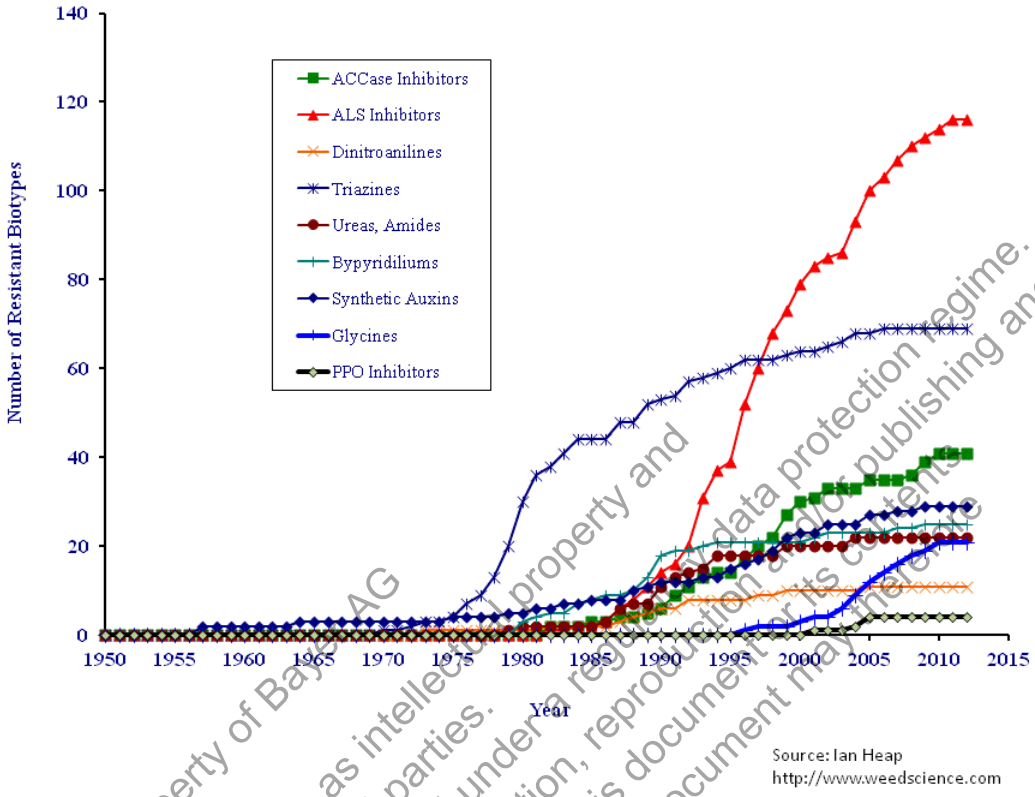

Figure I-1. Weed Resistance to Various Herbicide Families[1]

As can be seen in Figure I-1, weed resistance to the synthetic auxin group of herbicides has been slower to develop than for other herbicide groups even though these were the first synthetic herbicides discovered and used commercially. Possible reasons for this are discussed below.

[1]Global number of resistant biotypes

I.6. Mechanisms of Resistance and Inheritance of Resistance

To date, the three known basic mechanisms by which weed species develop resistance to a herbicide have been identified: 1) target site alteration (target site), 2) enhanced metabolism of the herbicides (metabolism), and 3) 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 alteration is the most common resistance mechanism among the various herbicide groups and chemical families. 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 other herbicide groups, such as glyphosate (Powles and Yu, 2010). The most common type of target site alteration is one where amino acid substitution(s) occur in the protein that is the target of the herbicide such that the alteration prevents the binding of the herbicide to the protein and as a result the activity of the targeted protein is not altered and the plant grows normally.

In the case of synthetic auxin herbicides, resistance has been speculated to be due to mutation(s) in genes encoding an auxin-binding protein causing reduced herbicide binding (Zheng and Hall, 2001; Goss and Dyer, 2003). In several studies, differential herbicide absorption, translocation, and metabolism were ruled out as possible mechanisms of resistance in kochia (Cranston et al., 2001) and in wild mustard (Zheng and Hall, 2001). However, current research has not presented convincing evidence for a single mechanism of resistance and this inability to elucidate the mechanism of resistance may be due to a lack of thorough understanding of the mechanism (mode) of action of auxin herbicides (Jasieniuk et al., 1996). Walsh et al. (2006) identified seven alleles at two distinct genetic loci that conferred significant resistance to picolinate auxins (picloram) in *Arabidopsis*, yet had minimal cross-resistance to 2,4-D and IAA, a naturally occurring plant growth regulator..

Multiple mechanisms for inheritance of dicamba resistance have been reported in the literature. Jasieniuk et al. (1995) reported results indicating that inheritance of dicamba resistance in wild mustard is determined by a single, completely dominant nuclear allele. However, Cranston et al. (2001) reported results indicating that dicamba resistance in kochia is determined by a quantitative trait (two or more genes). The slow development of weed resistance to synthetic auxin herbicides may in part be due to their proposed multiple sites of physiological action in plants (Jasieniuk et al., 1996) and to the possibility that inheritance, at least in some species, is determined by a quantitative trait (Cranston et al., 2001).

Little is known about the resistance mechanisms in glufosinate-resistant biotypes. Avila-Garcia and Mallory-Smith (2011) conducted an initial set of experiments to understand the mechanism of resistance in the ryegrass population that was also resistant to glyphosate. They found that resistance was not due to an insensitive or altered target site and hypothesized that reduced translocation is responsible for the resistance to both glyphosate and glufosinate in these populations.

I.7. Weeds Resistant to Dicamba and Glufosinate

As noted earlier, like other herbicides, the use of dicamba may lead to the development of dicamba-resistant weed species. To date, there are four species with known resistant biotypes to dicamba in the U.S./Canada after over 40 years of use: common hempnettle, kochia, prickly lettuce, and wild mustard (Heap, 2012a). Additionally, a population of common lambsquarters has been confirmed to be resistant in New Zealand, for a total of five species worldwide with confirmed resistant biotypes to dicamba. For the synthetic auxin group of herbicides there exist a total of 29 species globally with biotypes having confirmed resistance to at least one member of this group, but only nine species in the U.S. and four species in Canada (Heap, 2012a). All of these populations are, except for

two (wild carrot in OH and MI, and waterhemp in NE), found in western states or western Canadian provinces. In some weed species, cross-resistance between different herbicides within the auxin group has been confirmed (plant cross-resistance to another herbicide as a result of exposure to a similarly acting herbicide). Therefore, consideration has to be given to the possibility that dicamba resistance could extend to some of the other broadleaf species listed as resistant to other synthetic auxin herbicides (Cranston et al., 2001; Jasieniuk et al., 1995; Miller et al., 2001). However, because of differences in sites of action among the chemistry families within this group (*i.e.*, benzoic acids compared to pyridine-carboxylic acids) cross resistance between the herbicide groups is not a certainty (Monaco et al., 2002).

With the introduction of MON 88701 into glyphosate-tolerant cotton systems, where dicamba will be applied in combination with glyphosate and glufosinate, it is important to note that kochia is the only broadleaf species with resistant biotypes to either synthetic auxins or glyphosate. However, there are no known kochia biotypes resistant to both of these herbicides or resistant to glufosinate. In addition, the evolution of a dicamba-glyphosate resistant biotype is unlikely because dicamba, glyphosate, and/or glufosinate, each with a distinct mode-of-action, will likely be applied in the same season to MON 88701 in the glyphosate-tolerant cotton systems. If populations with resistance to both glyphosate and dicamba herbicides were to occur, there are other herbicide options for managing the weed in cotton (*e.g.*, glufosinate, clomazone and flumioxazin) and in its rotational crops (*e.g.*, atrazine and isoxaflutole in corn) (Table I-1). The glyphosate-resistant kochia biotype may be found in western cotton growing areas of Texas and Oklahoma.

To date there are two weed species with confirmed resistance to glufosinate: goosegrass in Malaysia and Italian ryegress in Oregon, U.S. (Heap, 2012d). In the case of goosegrass, the resistant populations evolved due to use of glufosinate in a rubber plantation (Seng et al, 2010). In the case of Italian ryegrass, the resistance was actually discovered in populations exposed to glyphosate that evolved resistance to glyphosate and which had not been exposed to glufosinate; exemplifying a case of cross-resistance (Avila-Garcia and Mallory-Smith, 2011). No resistance in a broadleaf species has been found to date.

Italian ryegrass may require special consideration when designing appropriate management programs because of the potential for cross resistance between glyphosate and glufosinate to exist. Avila-Garcia and Mallory-Smith (2011) demonstrated the only case of glufosinate cross resistance, which developed when the populations evolved resistance to glyphosate. It is not known if the reverse is true, though it is possible. Where there are known glyphosate resistant ryegrass populations Monsanto will recommend not to use glufosinate to control these populations. Likewise, dicamba will not be an option, since it does not control grasses such as ryegrass. Other herbicides such as those in the ACCase or ALS classes will be recommended. It is important to note that ryegrass is generally a weed target in preplant burndown applications and not in the cotton crop itself because of the biology of the species.

I.8. Sustainable Use of Dicamba and Glufosinate as a Weed Management Option in Cotton

MON 88701 will be sold only in cotton varieties that also contain other herbicide-tolerant traits, including glyphosate-tolerance. Cotton varieties containing both MON 88701 and a glyphosate-tolerant system will enable dicamba and glufosinate to be applied with glyphosate and/or other cotton herbicides in an integrated weed management program. Dicamba primarily will be used in mixtures with either glyphosate or glufosinate or in sequence with glyphosate or glufosinate to control a broad spectrum of grass and broadleaf weed species. Glyphosate and glufosinate will not be used in mixtures due to antagonism (*i.e.*, glufosinate damages the leaf tissue before glyphosate gets into the plant and/or can be translocated to growing parts of the plant) and reduced efficacy of glyphosate on susceptible weed species. Dicamba and glufosinate applications on MON 88701 will provide effective control of glyphosate-resistant broadleaf weeds and improve the control of annual and perennial broadleaf weed species, some of which are difficult to control with glyphosate. Dicamba and glufosinate will also help delay development and/or combat existing weed resistance issues that can limit the use of the PPO- and ALS-inhibiting herbicide groups by providing additional modes-of-action for management of certain broadleaf species known to be prone to resistance to many of the current herbicide options for weed management (*i.e.*, *Amarathus* spp.). Likewise, dicamba will help to mediate potential evolution of resistance to glufosinate in broadleaf species and glufosinate will do the same for the potential evolution of resistant broadleaf species to dicamba. Cultivation of a combined MON 88701 and glyphosate-tolerance trait product will foster the adoption of Integrated Pest Management (IPM) practices in cotton by allowing growers to continue to primarily focus on postemergence in-crop weed control, as they have practiced with the glyphosate-tolerant cotton systems. This will allow growers to delay some herbicide treatments until field scouting indicates a need for additional postemergence weed control which is consistent with the principles of IPM, and also herbicide resistance management practices. Increasing postemergence herbicide options in cotton is important, especially in conservation tillage situations, where consistency of postemergence herbicides has generally been greater than that of soil active residual products, which have greater degree of inconsistent weed control, and thus has been a factor in the adoption of conservation tillage systems in the U.S.

Upon the integration of MON 88701 into the glyphosate-tolerant cotton systems and pending approval of the use of dicamba on MON 88701 by the U.S. EPA, preplant/preemergence applications of dicamba can be made up to 1.0 lb a.e./acre up through crop emergence (cracking) and in-crop postemergence applications up to 0.5 lb a.e./acre could be applied through 7 days preharvest, with the combined total not to exceed 2.0 lbs a.e. dicamba per year for all applications. Residual herbicides also will be recommended for use, to provide early season weed control and to supplement dicamba and glufosinate activity on certain hard-to-control and glyphosate-resistant weed biotypes, such as glyphosate-resistant Palmer amaranth where weed populations can be very substantial. See section I.8.1 for specific weed management recommendations.

Dicamba and glufosinate, as complementary herbicides to glyphosate, will provide new weed control options in cotton that strengthen the utility and sustainability of glyphosate

as a weed control tool in the glyphosate-tolerant cotton systems. Likewise, glyphosate, as a complementary herbicide to dicamba and glufosinate, would strengthen the utility and sustainability of dicamba and glufosinate as weed control tools for the combined MON 88701 glyphosate-tolerance trait product.

In the event there is known or suspected presence of a dicamba-resistant or glufosinate-resistant weed biotype, other options for managing the resistant biotypes are available to the grower. There are multiple preemergence (including soil residuals) and postemergent herbicide options for managing weed populations that are resistant or may potentially develop resistance to dicamba or glufosinate in cotton, as well for crops grown in rotation with cotton. These options are noted in Table I-1.

Table I-1. Management Recommendations for Control of Dicamba-, Glufosinate- and Other Selected Synthetic Auxin-Resistant Weeds

Weed Species[1]	Herbicide Resistant Biotypes	Primary Crop Cotton	Rotational Crops			
			Corn	Sorghum	Soybeans	Wheat
Kochia	dicamba, fluroxpyr (populations also resistant to glyphosate)	Clomazone[a] Flumioxazin[i] Glyphosate[i] Paraquat[i]	Atrazine[a] Saflufenacil[a] Isoxaflutole[a] Mesotrione[a] Glyphosate[a]	Atrazine[a] Saflufenacil[a] Isoxaflutole[a] Mesotrione[a] Glyphosate[a]	Saflufenacil[a] Clomazone[a] Flumioxazin[a] Glyphosate[a] Paraquat[a]	Saflufenacil[a] Glyphosate[a] Bromoxynil/MCPA[a]
Prickly Lettuce	Dicamba, 2,4 D, MCPA	Glyphosate[i] Paraquat[i] Flumioxazin[i]	Saflufenacil[a] Atrazine[a] Carfentrazone + atrazine[a] Isoxaflutole + atrazine[a]	Saflufenacil[a] Atrazine[a] Carfentrazone + atrazine[a] Isoxaflutole + atrazine[a]	Saflufenacil[a] Chlorimuron/metribuzin[a] Glyphosate + imazethapyr[a]	Saflufenacil[a] Triasulfuron[a] Metsulfuron + thifensulfuron[a]
Wild mustard	Dicamba , 2,4 D,MCPA, picloram, dichlorprop, mecoprop	Glyphosate[i] Paraquat[i]	Glyphosate[c] Atrazine[c] Primisulfuron[c] Nicosulfuron[d] Halosulfuron[d]	Glyphosate[c] Atrazine[c] Primisulfuron[c] Nicosulfuron[d] Halosulfuron[d]	Glyphosate[c] Chlorimuron[c] Chlorimuron/metribuzin[c]	
Field Bindweed	2,4 D	Glyphosate[i] Paraquat[i] Flumioxazin[i]	Glyphosate[a] Glyphosate + imazethapyr[a] Glyphosate + Imazamox[a]	Glyphosate[a] Glyphosate + imazethapyr[a] Glyphosate + Imazamox[a]	Glyphosate[a]	Glyphosate[a]
Yellow Starthistle[e]	Picloram					

Table I-1. Management Recommendations for Control of Dicamba-, Glufosinate- and Other Selected Synthetic Auxin-Resistant Weeds (continued)

Weed Species[1]	Herbicide Resistant Biotypes	Primary Crop Cotton	Rotational Crops			
			Corn	Sorghum	Soybeans	Wheat
Spreading Dayflower	2,4 D					Bentazon halosulfuron penoxsulam bispyribac[f]
Lambsquarters[g]	Dicamba	Paraquat[i] Flumioxazin[i] Glyphosate[b]		Isoxaflutole[a] Atrazine[a] Saflufenacil[a] Mesotrione[a] Bromoxynil[b]	Metribuzin[b] Cloransulam[b] Saflufenacil[a] Imazamox[b] Glyphosate[b]	Bromoxynil[a] Chlorsulfuron/Metsulfuron[a] Glyphosate[a] Saflufenacil[a]
Goosegrass	Glufosinate	Clethodim[h] Glyphosate[h] pendimethalin[h] trifluralin[h]	Glyphosate[h] pendimethalin[h]	Glyphosate[h]	Clethodim[h] Glyphosate[h] pendimethalin[h] trifluralin[h]	Glyphosate[h]
Italian ryegrass	Glufosinate (populations also resistant to glyphosate)	Metolachlor (fall applied)[h] Clethodim[h] Glyphosate[h] Paraquat[i]	Metolachlor (fall applied)[h] Glyphosate[h]	Metolachlor (fall applied)[h] Glyphosate[h]	Metolachlor (fall applied)[h] Clethodim[h] Glyphosate[h]	Glyphosate[h]

[1]Scientific names for each weed species can be found in Table VIII-4.
[a][redacted] et al., 2010.
[b][redacted] et al., 2010.
[c]MSU, 2010.
[d]Kells and Stachler, 1997.
[e]PNWE, 2010.
[f]University of Arkansas CES, 2010.
[g]Resistance to lambsquarters has only been confirmed in New Zealand.
[h][redacted] et al., 2011
[i]Smith et al., 2012

I.9. Stewardship of Dicamba and Glufosinate Use on MON 88701

In order to steward the use of agricultural herbicides and herbicide-tolerant cropping systems such as the combined trait MON 88701 and glyphosate-tolerant cotton product, Monsanto has conducted investigations and worked extensively with academics and other herbicide manufacturers to understand and recommend best practices to manage herbicide resistance. These investigations have demonstrated that one of the major factors contributing to the development of resistant weed biotypes has been poor weed control management practices. The primary reasons for lack of adequate management includes: 1) application of herbicides at rates below those indicated on the product label for the weed species, and 2) sole reliance on a particular herbicide for weed control without the use of other herbicides or cultural control methods (Beckie, 2006; [redacted] et al., 2007).

I.9.1. Weed Control Recommendations

The proposed label for dicamba use on MON 88701 is based on the maximum allowable use rates and patterns. Prior to launch of MON 88701 in glyphosate-tolerant cotton systems, Monsanto, in cooperation with academics, will conduct trials to confirm the optimum rate and timing for dicamba, glufosinate and glyphosate, alone and in combination, and other herbicides. Recommendations to growers will be developed from this information and will be provided in herbicide product labels, Monsanto's Technology Use Guide (TUG), and in other education and training materials to be broadly distributed. Specifically, current research conducted by Monsanto to define the optimum weed management systems support use recommendations that include the application of dicamba and glyphosate for preemergence on conservation tillage acres and early postemergence in-crop applications. In some situations, a second in-crop application of either dicamba tank-mixed with glyphosate or glufosinate, with or without a soil residual will be recommended (see Section VIII.G.4 for additional details)

These recommendations will ensure more than one mechanism of action against the targeted species, which is a fundamental component of a good weed resistance management program. These management systems, which include the use of multiple effective herbicide modes-of-action, will reduce the potential for further resistance development to glyphosate, dicamba, and glufosinate, as well as other critical cotton herbicides. Furthermore, the preplant weed spectrum is generally different from the in-crop weed spectrum therefore multiple applications of glyphosate and dicamba are not expected to increase selection pressure on either herbicide.

I.9.2. Dispersal of Technical and Stewardship Information

Monsanto will use multiple methods to distribute technical and stewardship information to growers, academics and grower advisors. Monsanto's TUG will set forth the requirements and best practices for the cultivation of MON 88701 including recommendations on weed resistance management practices. Growers who purchase

varieties containing MON 88701 will be required to enter into a limited use license with Monsanto and must sign and comply with the Monsanto Technology Stewardship Agreement (MTSA), which requires the grower to follow the TUG.

The weed resistance management practices that will be articulated in the TUG will also be broadly communicated to growers and retailers in order to minimize the potential for the development of resistant weeds. These practices will be communicated through a variety of means, including direct mailings to each grower purchasing a cotton variety containing MON 88701, a public website[9], and reports in farm media publications. The overall weed resistance management program will be reinforced through collaborations with U.S. academics, who will provide their recommendations for appropriate stewardship of dicamba and glufosinate in cotton production, as well as by collaboration with crop commodity groups who have launched web-based weed resistance educational modules. Finally, Monsanto will urge growers to report any incidence of repeated non-performance of dicamba or glufosinate on weeds in fields planted with MON 88701, and Monsanto will investigate cases of unsatisfactory weed control to determine the cause as defined in I.9.

The EPA is the U.S. federal regulatory agency that administers the federal law governing pesticide sale and use under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). EPA encourages pesticide manufacturers to provide growers with information regarding an 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 Pesticide Registration (PR) Notice 2001-5 (U.S. EPA, 2001). 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 approves all pesticide label use instructions based on its evaluation of supporting data supplied by the pesticide registrant or manufacturer. By approving a label, EPA has concluded that the product will not cause unreasonable adverse effects to the environment when used in accordance with the label's directions. 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 agricultural herbicide labels, and will continue to do so in the future. Monsanto will adopt a similar approach to pesticide resistance management guidance on its dicamba product labels.

In summary, Monsanto will require weed resistance management practices through the MTSA and TUG for its biotechnology-derived herbicide-tolerant products, such as MON 88701 integrated into the glyphosate-tolerant cotton systems, and to promote these practices through product labeling and educational outreach efforts as an effective means to manage weed resistance development for both dicamba, glufosinate, and glyphosate.

[9] http://www.monsanto.com/weedmanagement/Pages/default.aspx

I.9.3. Weed Resistance Management Practices

Monsanto will provide information to growers and grower advisors on best management practices to delay the development of resistance to dicamba and glufosinate. The weed resistance management recommendations for the use of dicamba and glufosinate in conjunction with cotton varieties containing MON 88701 will be consistent with the Herbicide Resistance Action Committee's guidelines for prevention and management of herbicide resistance (HRAC, 2010)[10]. These guidelines recommend an integrated approach to weed resistance management, including crop management (*i.e.*, cover crops, crop rotation, etc.), cultivation techniques, and the use of multiple herbicide modes-of-action to manage a weed population.

In cases where resistance is confirmed for dicamba or glufosinate in cotton producing areas, Monsanto and University/Cooperative Extension Service (CES) personnel will provide recommendations for alternative herbicide control methods to growers. These recommendations would be made available through Monsanto supplemental labels, Monsanto and university publications, and internet sites to growers, consultants, retailers and distributors. For all existing cases of dicamba-resistant and glufosinate-resistant weeds in the U.S. and globally today, alternative herbicides and cultural methods are available to growers to effectively control these biotypes. Examples of recommended alternative herbicides from University/CES personnel that are applicable to weed species known to be resistant to glufosinate, dicamba and other synthetic auxin herbicides are found in Table I-1. However, these examples in Table I-1 are only a subset of product combinations of available cotton herbicides.

I.10. Monsanto Weed Performance Evaluation and Weed Resistance Management Plan

An important part of a weed resistance management plan is the timely acquisition of information regarding product performance. Monsanto has an extensive technical, sales and marketing presence in the cotton markets where MON 88701 will be grown. Through our relationships with farm advisors, key University/CES personnel, and growers using our seeds and traits products, Monsanto will acquire important and timely information regarding product performance. This will allow the timely recognition of performance issues that could arise related to weed resistance or other means. Field employees and hired consultants are trained and provided processes for responding to product performance inquiries. Individual performance issues that could be related to potential resistance are promptly handled. In addition performance inquiries are periodically reviewed by Monsanto for trends that could indicate the need for follow up action on a broad scale.

If dicamba or glufosinate resistance is confirmed, the scientific and grower communities will be notified and a weed resistance mitigation plan will be implemented by Monsanto

[10] The Herbicide Resistance Action Committee (HRAC) is an international body founded by the agrochemical industry for the purpose of supporting a cooperative approach to the management of herbicide resistance and the establishment of a worldwide herbicide resistance database.

in cooperation with the University/CES and/or the appropriate herbicide producer . The mitigation plan will be 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: 1) biology and field characteristics of the weed (seed shed, seed dormancy, etc.), 2) importance of the weed in the agricultural system, 3) resistance status of the weed to other herbicides with alternate modes-of-action, and 4) 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 herbicide labeling (labeling which includes newly approved use directions, or other instructions)[11], informational fact sheets, retailer training programs, agriculture media and/or other means, as appropriate.

In addition to the grower inquiry initiated process, Monsanto, alone and/or in cooperation with University/CES, will conduct field studies to understand the potential for weed resistance and weed shifts as the result of various weed management programs implemented for MON 88701 integrated into glyphosate-tolerant cotton systems. These studies will allow researchers to better track specific factors that can influence the development of resistance to specific weeds.

I.11. Summary

Development of weed resistance is a complex process that can be difficult to accurately predict. Multiple methods for managing weed resistance are available and no single option is best for all farming situations. 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, tailored for the particular herbicide and weed species, and utilize an integrated system approach 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 products will allow dicamba and glufosinate herbicides to continue to be used effectively. In cases where weed populations have evolved or developed resistance to dicamba and/or glufosinate, effective management options are available and experience has shown that growers will continue to find value in using dicamba and glufosinate in their weed control programs.

The key principles for effective stewardship of dicamba and glufosinate use, including the integration of MON 88701 in the glyphosate-tolerant cotton systems, comprise:

[11] Monsanto will communicate information broadly so registrants are aware of when Monsanto is not the registrant or provider of the chemistry,.

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.

Overall, there is a low potential for dicamba-resistant broadleaf weed populations to arise from the use of dicamba applied to MON 88701 integrated into glyphosate-tolerant cotton systems. The reasons are as follows:

- Dicamba will be used in combination with glyphosate and/or glufosinate in a majority of cropping situations, and weed recommendations will also include the concurrent use of residual herbicides for complementary weed control and different modes-of-action. These use patterns mean that there will be multiple modes-of-action against the major broadleaf species present in cotton production. This is a primary way to delay the development of resistance.
- The development of resistance to auxin herbicides has been found to be relatively slow. This observation is hypothesized to be due to multiple sites of action within plants and evidence suggesting that resistance is determined by multiple genes (quantitative traits), at least in some species.
- Only four broadleaf weed species have been confirmed to be resistant to dicamba in the U.S., and relatively low numbers of broadleaf species have been confirmed to be resistant to synthetic auxin herbicides even though dicamba has been widely in use for over 40 years.
- Known resistant broadleaf populations to dicamba and other auxin herbicides are primarily found in the western U.S. and, thus, are not present in the major cotton geographies. In addition, the known dicamba-resistant biotypes are not major weed species present in the U.S. cotton crop.

Likewise, the probability for weed species to evolve resistance to glufosinate as a result of glufosinate use in the MON88701 system is considered to be low because:

- Two species have been confirmed to be resistant to glufosinate worldwide and one (ryegrass) in the US. This suggests that the frequency for resistant alleles in native weed populations is fairly low.
- Known resistant populations to glufosinate herbicide within the U.S. are only found in Oregon, and thus, are not present in the major cotton geographies.
- In the MON 88701 system, glufosinate will likely be used in combination with dicamba and in sequence with glyphosate. Residual herbicides will also be recommended and likely used in this cropping system. As noted above, these use patterns mean that there will be multiple modes-of-action against the major broadleaf species present in soybean production. This is a primary way to delay the development of resistance.

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