

Monsanto Petition (12-185-01p) for Determination of Nonregulated Status of Dicamba and Glufosinate Resistant MON 88701 Cotton

OECD Unique Identifier:
MON-88701-3

Plant Pest Risk Assessment

April 2014

Agency Contact
Cindy Eck
Biotechnology Regulatory Services
4700 River Road
USDA, APHIS
Riverdale, MD 20737
Fax: (301) 734-8669

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, sexual orientation, or marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA'S TARGET Center at (202) 720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 1400 Independence Avenue, SW, Washington, DC 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

Mention of companies or commercial products in this report does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

This publication reports research involving pesticides. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

TABLE OF CONTENTS

PAGE

A.	Introduction.....	1
B.	Development of MON 88701 Cotton	3
C.	Expression of the Gene Product and Changes to Plant Metabolism.....	6
D.	Potential Impacts on Disease and Pest Susceptibilities	10
E.	Potential Impacts on Nontarget Organisms Beneficial to Agriculture	14
F.	Potential for Enhanced Weediness of MON 88701	16
G.	Potential Impacts on the Weediness of Any Other Plants with which It Can Interbreed.....	18
H.	Potential Changes to Agriculture or Cultivation Practices	22
I.	Potential Impacts from Transfer of Genetic Information to Organism with which MON 87701 Cannot Interbreed	24
J.	Conclusion	26
K.	References.....	26

A. Introduction

Monsanto Company (referred hereafter to as Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) for a determination that the genetically engineered (GE) dicamba and glufosinate herbicide-resistant¹ cotton event MON 88701 (hereafter referred to as MON 88701) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 12-185-101p, and is hereafter referenced as Monsanto 2012. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq.*)². This plant pest risk assessment was conducted to determine if MON 88701 is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs any genera or taxa designated in 7 CFR § 340.2 and is also considered a plant pest. A GE organism is also regulated under 7 CFR part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have sufficient information to determine if the GE organism is unlikely to pose a plant pest risk. MON 88701 was produced by *Agrobacterium*-mediated transformation and includes introduced genetic sequences derived from plant pest organisms listed in 7 CFR § 340.2 (Monsanto 2012). Monsanto has conducted introductions of MON 88701 as a regulated article under APHIS-authorized notifications since 2007 (Table A-1, pages 264-265 in Monsanto 2012), in part, to gather information to support that it is unlikely to pose a plant pest risk.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with MON 88701 and its progeny and their use in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 88701 is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR § 340.6(c) specify the information needed for consideration

¹ Monsanto has described the phenotype of MON 88701 as "herbicide tolerant" and historically APHIS has also referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America's definition of "herbicide resistance" since MON 88701 has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type" (WSSA, 1998). By the WSSA definition, "resistance [to an herbicide] may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

² The Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, changes to agricultural or cultivation practices that may impact diseases and pests of plants, effects of the regulated article on nontarget organisms, indirect plant pest effects on other agricultural products, and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, June 26, 1986). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with the APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

The EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. The EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA). Dicamba is currently labeled for use on cotton only as a preplant application, due to its ability to injure cotton (BASF 2010; U.S. EPA 2009). According to Monsanto, it is currently used on approximately 8% of cotton acreage, and is a leading herbicide for use against glyphosate-resistant marestail (horseweed) in the Midsouth region (McClelland *et al* 2006). Monsanto will submit an application to amend EPA Registration Number 524-582 to allow preemergence and in-crop postemergence application of dicamba on MON 88701, an increase in the dicamba residue tolerance for cottonseed from 0.2 ppm to 3 ppm, the establishment of a tolerance of 70 ppm for cotton gin by-products, and the inclusion of the herbicidally inactive dicamba metabolite 3,5-dichlorosalicylic acid (DCSA) in the residue definitions for both cottonseed and gin by-products (page 36 in Monsanto 2012). Glufosinate is currently labeled for use on glufosinate-tolerant cotton from emergence through the early bloom growth stage (Bayer CropScience 2011; U.S. EPA 2003; page 37 in Monsanto 2012). It is currently undergoing reregistration at EPA with the Reregistration Eligibility Decision expected by the end of 2013 (U.S. EPA 2008). According to Monsanto, it is currently used on approximately 8% of cotton acreage (Table VIII-9, page 193, in Monsanto 2012). The use pattern and rate of glufosinate on MON 88701 will follow the exiting glufosinate-tolerant cotton uses outlined on the glufosinate herbicide label, and glufosinate residues in MON 88701 treated with glufosinate are below the EPA-established residue tolerances (page 37 in Monsanto 2012). Therefore, Monsanto will not seek changes in existing glufosinate labels or the established tolerances for its use on MON 88701 (page 37 in Monsanto 2012).

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary consultation process. Monsanto submitted a food and feed safety and compositional assessment to FDA (BNF No. 135, April 2012) (page 36 in Monsanto 2012).

B. Development of MON 88701 Cotton

Cotton belongs to the genus *Gossypium*, which consists of approximately 50 species, four of which are generally cultivated in tropical and subtropical regions around the world (Fryxell 1984; OECD 2008; Percival *et al* 1999). The most commonly cultivated species in the United States, *G. hirsutum* (Upland cotton), comprises 98% of the cotton crop (USDA-NASS 2012a) and is the subject of this risk assessment. Over the last decade, it has been grown on an average of 12.2 million acres annually in 17 states from Virginia southward and westward to California - an area often referred to as the Cotton Belt (Figure 1). Smaller amounts of *G. barbadense* (Pima or Egyptian cotton) are cultivated in Arizona, California, New Mexico, and Texas (USDA-NASS 2012a; Pleasants and Wendel 2005). Cotton is a perennial plant cultivated as an annual, and is more limited geographically than other major crops in the United States because it can be grown only in regions with more than 180 frost-free days per year. Two other cultivated species, *G. arboreum* and *G. herbaceum*, are not grown in the U.S. or its territories.

Weed control in cotton is essential to maximize both cotton fiber yield and quality. Historically, mechanical tillage and hand hoeing were the primary means of controlling weeds in cotton. Herbicide use began to develop in the 1940s and 1950s, and rapidly accelerated in the 1960s as a series of more selective herbicides were introduced into the market. By the mid-1980s, over 30 herbicides and herbicide combinations were being used in cotton (Buchanan 1992; pages 187-188 in Monsanto 2012). With the commercial introduction of glyphosate resistant cotton in 1997, glyphosate use increased while the use of other herbicides decreased (Webster and Nichols, 2012). According to Monsanto, glyphosate is now used on approximately 90% of cotton acres grown (Table VIII-9, pages 192-193 in Monsanto 2012). Although glufosinate resistant cotton was introduced in 2003, as of 2010 it was planted on only 3% of cotton acres (USDA-ERS-FAS 2010; page 189 in Monsanto 2012).

Repeated and intensive use of herbicides with the same mechanisms of action can rapidly select for tolerant, difficult-to-control weeds and for herbicide-resistant weeds, especially in the absence of the concurrent use of herbicides with different mechanisms of action and/or use of different mechanical or cultural practices for weed control (Vencill *et al* 2012). Fourteen confirmed glyphosate-resistant weeds are now known in the U.S. (Heap 2012), including Palmer amaranth and waterhemp, two of the most troublesome weed species in cotton (Webster and Nichols 2012).

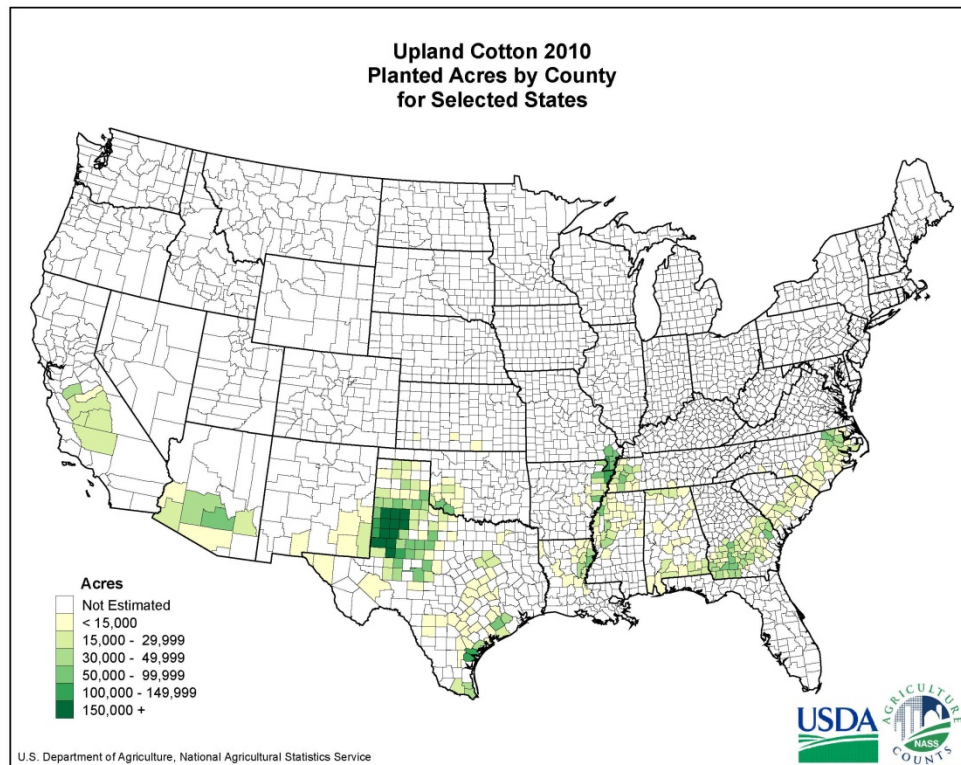


Figure 1. Cotton production areas in the U.S. (USDA-NASS 2012b)

MON 88701 is an Upland cotton variety that has been genetically engineered to be resistant to the herbicides dicamba and glufosinate. Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective pre- and post-emergent herbicide used to control a wide spectrum of broadleaf weeds and woody plants, while glufosinate is a broad-spectrum pre- and post-emergent contact herbicide that provides nonselective control of a wide range of broadleaf and grass weeds (BASF 2010; Bayer CropScience 2011; page 34 in Monsanto 2012). According to Monsanto, MON 88701 was developed to provide a wider window of application for dicamba beyond current pre-plant cotton uses and to enable improved control of a broad spectrum of weed species in cotton crops, including glyphosate resistant weeds and weeds that are difficult to control using glyphosate or other herbicides, in a manner that will impede and delay the emergence of further herbicide resistance in weeds (pages 4-5, 34-35, and 206-207 in Monsanto 2012). MON 88701 will allow in-crop applications of dicamba from preemergence to seven days preharvest. It will also allow in-crop applications of glufosinate from emergence through the early bloom growth stage, as is done with currently marketed glufosinate resistant cotton. Monsanto will use traditional breeding methods to combine MON 88701 with deregulated, glyphosate-resistant cotton events to allow the use of herbicides with three distinct modes of action in an integrated weed management program (pages 35, 209, and 212 in Monsanto 2012).

MON 88701 was generated via transformation of hypocotyl segments from the conventional variety Coker 130 (Bowman *et al* 2006) using a disarmed strain of *Agrobacterium tumefaciens*, to express the dicamba monooxygenase (DMO) and phosphinothricin acetyltransferase (PAT) proteins. Following transformation, the hypocotyl segments were grown on medium containing glufosinate to eliminate untransformed cells and antibiotics to eliminate the bacterial vector, and were regenerated into whole plants (pages 40-41 in Monsanto 2012). The DMO protein, encoded by the *dmo* gene from *Stenotrophomonas maltophilia*, confers resistance to dicamba-containing herbicides by demethylating dicamba to the herbicidally inactive metabolites DCSA and formaldehyde (pages 77-78 in Monsanto 2012). The PAT protein, encoded by the *bar* (bialaphos resistance) gene of *Streptomyces hygroscopicus*, confers resistance to glufosinate-containing herbicides by acetylating the free amino group of glufosinate to produce non-herbicidal N-acetyl glufosinate (pages 81-82 in Monsanto 2012).

Description of the genetic modifications

As described in the petition (pages 40-48 in Monsanto 2012), MON 88701 was produced by *Agrobacterium*-mediated transformation of Coker 130 with the binary plasmid vector PV-GHHT6997. The plasmid generates a T-DNA that contains two gene expression cassettes flanked by left and right border regions and non-coding sequences used in DNA cloning, and separated by a short (12 base pair) intervening sequence.

The *dmo* gene expression cassette for production of the DMO protein contains the following genetic elements:

- Promoter: PC1SV from the full-length transcript of peanut chlorotic streak caulimovirus (Maiti and Shepherd 1998)
- Intervening sequence: Short segment (6 base pairs), used in DNA cloning
- Leader: 5' untranslated region (UTR) leader sequence from the RNA of tobacco etch virus (Niepel and Gallie 1999)
- Intervening sequence: One base pair, used in DNA cloning
- Targeting sequence: coding sequence for the chloroplast transit peptide CTP2 derived from the *CTP2* target sequence of the *shkG* gene from *Arabidopsis thaliana* (Herrmann 1995; Klee *et al* 1987)
- Gene: *dmo* codon-optimized coding sequence for the DMO protein of *S. maltophilia* (Herman *et al* 2005; Wang *et al* 1997), with an insertion of a single codon (encoding the amino acid leucine at position 2 of the protein) (page 79 in Monsanto 2012)
- Intervening sequence: short segment (54 base pairs), used in DNA cloning
- Terminator: 3' UTR sequence from the *E6* gene of *G. barbadense* (John 1996)

The *bar* expression cassette for production of the PAT protein contains the following genetic elements:

- Promoter: e35S from the 35S RNA of cauliflower mosaic virus containing a duplicated enhancer region (Kay *et al* 1987; Odell *et al* 1985)
- Intervening sequence: short segment (3 base pairs), used in DNA cloning

- Leader: 5' UTR of the *DnaK* gene from *Petunia hybrida* (Rensing and Maier 1997; Winter *et al* 1988)
- Intervening sequence: short segment (6 base pairs), used in DNA cloning
- Gene: *bar* coding sequence for the PAT protein of *S. hygroscopicus* (Thompson *et al* 1987)
- Intervening sequence: short segment (19 base pairs), used in DNA cloning
- Terminator: 3' UTR sequence of the *nos* gene from *A. tumefaciens* pTi (Bevan *et al* 1983; Fraley *et al* 1983)

Monsanto provided evidence demonstrating that,

- the DNA inserted into the MON 88701 genome is present at a single locus and contains one functional copy each of the *dmo* and *bar* genes (Figure IV-1, page 53, Table IV-1, page 54, & Figures IV-2 – IV-4, pages 60-62 in Monsanto 2012);
- the right border sequence and a portion of the right side non-coding sequence, as well as a portion of the left border sequence, are deleted in MON 88701 while all other genetic elements in the insert are intact as in the plasmid PV-GHHT6997 (page 51 and Figure IV-6, page 66 in Monsanto 2012);
- the final product does not contain any of the backbone sequences from PV-GHHT6997 (Figure IV-5, page 64 in Monsanto 2012);
- the inserted DNA was stably inherited across five generations (Figures IV-8 and IV-9, pages 70-71 in Monsanto 2012); plants that contain the inserted DNA also express the glufosinate herbicide resistance phenotype (as determined through glufosinate herbicide application), and the inserted DNA and resistance trait both segregate according to Mendel's laws of segregation consistent with the finding of a single insertion locus (Figure IV-10 and Tables IV-3 and IV-4, pages 74-75, in Monsanto 2012).

In addition, Monsanto stated that there was a small, 123 base pair deletion from the conventional genomic DNA at the T-DNA insertion site (page 67 in Monsanto 2012). Minor deletions and/or insertions of DNA are not uncommon during *Agrobacterium*-mediated transformation (Salomon and Puchta 1998).

C. Expression of the Gene Product and Changes to Plant Metabolism

USDA-APHIS assessed whether changes in plant metabolism or composition in MON 88701 is likely to alter its plant pest risk. The assessment encompasses a consideration of the expressed proteins and their effect on plant metabolism and an evaluation of whether the nutrients and anti-nutrient levels in harvested seed derived from MON 88701 are comparable to those in the conventional cotton control Coker 130 or to other reference cotton cultivars considered for the composition analysis. Forage is not considered, as harvested vegetative cotton biomass is never used as forage. Host plant quality (including such components as carbon, nitrogen, amino acid sources, trace elements, and defensive metabolites) is known to affect herbivore performance and fecundity; and higher-trophic level interactions, such as the performance of predators and parasitoids, may also be affected (reviewed by Awmack and Leather 2002). Similarly a wide array of secondary metabolites in plants is known to provide defense against microbes (Dixon

2001). Thus changes in host plant quality may have the potential to affect the ability of MON 88701 to withstand attack by pests and diseases or have impacts non-target organisms including beneficial organisms.

The DMO protein expressed in MON 88701 is a variant of the DMO protein encoded by the *dmo* gene of *S. maltophilia*. The *dmo* gene used was isolated from a stormwater retention pond outside a dicamba manufacturing plant (Krueger *et al* 1989). *S. maltophilia* is a gram negative bacterium that is ubiquitous in the environment and found associated with the rhizosphere of plants (Echemendia 2010; Palleroni and Bradbury 1993). It has been used as a biocontrol agent for fungal plant pathogens and is not known to cause disease in plants (Berg *et al* 1999). The DMO protein expressed in MON 88701 is 349 amino acids in length and has an apparent molecular weight of 39.5 kDa (Figure C-2, page 285, and Table C-4, page 291 in Monsanto 2012). It differs from the *S. maltophilia* DMO protein in that it includes an additional leucine at position two and an additional nine amino acids from CTP2 at its N-terminus that result from alternative processing of the transfer peptide (pages 79 and 275-276 in Monsanto 2012). Alternative processing of the CTP sequence has been previously observed (Behrens *et al* 2007; Clark and Lamppa 1992), and these differences should not have an effect on the structure, activity, or specificity of the MON 88701 DMO protein because the N-terminus and residue two are sterically distant from the catalytic site of the enzyme (D'Ordine *et al* 2009; Dumitru *et al* 2009). Functional assays demonstrated that the MON 88701 DMO protein was active and protected MON 88701 from damage following dicamba application (Tables C-5 and C-7, pages 297 and 299, in Monsanto 2012). The MON 88701 DMO protein was found to be expressed throughout the life-cycle and tissues of the plant, including roots and seed, with the highest level of expression in leaves (Table V-2, page 85 in Monsanto 2012). This is expected since expression of the *dmo* gene in MON 88701 is driven by the constitutive PC1SV promoter from peanut chlorotic streak caulimovirus (Maiti and Shepherd 1998).

DMO is an enzyme that confers herbicide resistance by catalyzing the demethylation of dicamba to the non-herbicidal compounds DCSA and formaldehyde (pages 77-78 in Monsanto 2012; Chakraborty *et al* 2005). In *S. maltophilia*, DMO is the terminal component of a three component system comprised of a reductase, a ferredoxin, and an oxygenase (DMO). These enzymes work together to transport electrons from nicotinamide adenine dinucleotide (NADH) to oxygen and catalyze the demethylation of an electron acceptor substrate. In MON 88701, the DMO protein is targeted to chloroplasts via the CTP2 sequence for co-localization with the endogenous reductase and ferredoxin enzymes that supply electrons for the DMO demethylation reaction (Behrens *et al* 2007). The DMO protein expressed in MON 88701 is highly specific for dicamba. *In vitro* assays using plant produced and *E. coli* produced MON 88701 DMO demonstrated that five endogenous metabolites and the herbicide 2,4-D, all of which are structurally similar to dicamba, were not metabolized by the enzyme (pages 79-80 and 301-306 in Monsanto 2012). In addition, *in vivo* assays demonstrated that, of 10 different herbicides applied, MON 88701 and control Coker 130 cotton exhibited similar levels of injury for all but dicamba, indicating that the other nine herbicides do not serve as a substrate for MON 88701 DMO (pages 80-81 and 297-299 in Monsanto 2012).

Therefore, because of the apparent high substrate specificity, the DMO protein is unlikely to catalyze the conversion of other endogenous substrates and thereby affecting the metabolic system of MON 88701.

DCSA is a known metabolite of dicamba whose safety in soybean, soil, and livestock has been evaluated by the EPA (U.S. EPA 2009; 40 CFR part 180.227 [7-1-11 Edition]) and whose structure is similar to salicylic acid (2-hydroxybenzoic acid), an endogenous plant benzoic acid (Frear 1976; p. 273 in Monsanto 2012). Because endogenous salicylic acid compounds are known to be involved in plant responses to stress, including to pests and pathogens (Bi *et al* 1997; Colson-Hanks *et al* 2000; Inbar *et al* 2001; Martinez *et al* 2000; Thaler *et al* 2010; Thaler *et al* 2012; Vlot *et al* 2009; Zarate *et al* 2007; Zhang *et al* 2011), the possible effect of DCSA production on host defense in MON 88701 will be further discussed in the later section on *Potential Impacts of Genetic Modifications on Pest and Disease Susceptibilities*. Formaldehyde has also been associated with plant stress responses, although its role if any in such responses is unclear (Sardi *et al* 1996; Szende and Tyihak 2010). In MON 88701, dicamba-derived formaldehyde is expected to be produced in small amounts; the maximum theoretical amount is estimated to be 6.3 – 33 mg/kg based on an assumption that the entire amount of dicamba applied to MON 88701 is intercepted and instantaneously metabolized by DMO (page 274 in Monsanto 2012). This is well within the range of formaldehyde measured in a variety of dicot plants (up to several hundred mg/kg) (Adrian-Romero *et al* 1999) and agricultural commodities (WHO-IPCS 1989). Moreover, any additional formaldehyde that would be produced in MON 88701 by dicamba treatment would be quickly metabolized and incorporated into the 1-carbon pool of the plant through known pathways (Hanson and Roje 2001; Kalasz 2003). Thus, the incremental increase in formaldehyde over and above the levels already presumed to be present in the cotton plant would likely be small and transient.

The PAT protein expressed in MON 88701 is encoded by the *bar* gene of *S. hygroscopicus* (Thompson *et al* 1987), a saprophytic bacterium that is widespread in the environment and is not pathogenic to plants (Kämpfer 2006; Kutzner 1981; Locci 1989). A homologous protein is encoded by the *bar* gene of *S. viridochromogenes* (Wohlleben *et al* 1988). The PAT protein expressed in MON 88701 is 183 amino acids in length and has an apparent molecular weight of 24.1 kDa (Figure C-10, page 315, and Table C-13, page 321, in Monsanto 2012). It differs from the *S. hygroscopicus* DMO protein by the deletion of one or two N-terminal amino acids (Table C-10, page 311, in Monsanto 2012). These differences should not affect the structure, activity, or specificity of the MON 88701 PAT protein because these amino acids are sterically distant from the catalytic site of the enzyme, based on the structure of a related acetyltransferase from *Pseudomonas aeruginosa* (Davies *et al* 2007). Functional assays demonstrated that the MON 88701 PAT protein was active and protected MON 88701 from damage following glufosinate application (Table C-14, page 327, and Tables IV-3 and IV-4, page 75, in Monsanto 2012). The MON 88701 PAT protein was found to be expressed throughout the life-cycle and tissues of the plant, including roots and seed, with the highest level of expression in seed (Table V-3, page 87, in Monsanto 2012). This is expected since expression of the *bar* gene in MON 88701 is driven by the constitutive e35S promoter from cauliflower mosaic virus (Kay *et al* 1987; Odell *et al* 1985).

PAT is an enzyme that confers herbicide resistance by catalyzing the acetylation of L-phosphinothricin, the active component in glufosinate, to the non-herbicidal compound N-acetyl L-phosphinothricin (Thompson *et al* 1987; Wehrmann *et al* 1996; pages 81-82 in Monsanto 2012). PAT proteins are highly specific for L-phosphinothricin. Other L-amino acids, including the L-phosphinothricin analogue L-glutamate, are unable to be acetylated by PAT and do not inhibit acetylation of L-phosphinothricin in competition assays (Wehrmann *et al* 1996). Therefore, the PAT protein is unlikely to affect the metabolic system of MON 88701. Numerous glufosinate resistant crops containing the PAT protein encoded by the *bar* gene have undergone regulatory review and approval in the United States and several other countries (see list in ILSI-CERA 2011; OECD 1999, 2002a). The safety of the protein has been established in the scientific literature (Herouet *et al*, 2005).

Monsanto carried out a compositional assessment comparing MON 88701 seed to seed from conventional control varieties using the principles outlined in the OECD consensus document on compositional considerations for cotton (OECD 2009). The samples for compositional assessment were collected from eight locations in 2010, chosen to represent typical cotton growing regions of the United States (page 99 in Monsanto 2012). Analytes were assessed quantitatively and included 47 nutrients (proximates, fiber, amino acids, fatty acids, minerals, and vitamin E) and five anti-nutrients (fatty acids, gossypol). Analyte levels in untreated MON 88701 and MON 88701 treated with both glufosinate (0.5 lbs acid equivalent/acre at the 3-5 leaf stage) and dicamba (0.5 lbs acid equivalent/acre at the 6 – 10 leaf stage) were compared to corresponding levels in the parental Coker 130 control (untreated) and to statistical tolerance intervals generated from nine non-GE commercial varieties grown concurrently at the same field sites (four commercial varieties per site) (page 99 and Table E-1, page 337, in Monsanto 2012). The latter group was included in the analysis to establish a spectrum of normal variation for the measured analytes in cotton. Statistical analysis to detect significant differences in analyte levels was performed using data collected from individual field sites as well as the combined set of data collected across all field sites.

Statistically significant ($p < 0.05$) differences in the combined-site nutrient levels were observed for 16 nutrients in both herbicide treated and untreated MON 88701 compared to the Coker 130 control: the proximates ash, calories, carbohydrates, moisture, and total fat; acid detergent fiber, neutral detergent fiber, total dietary fiber; the amino acid arginine; the fatty acid 14:0 myristic acid; the minerals calcium, magnesium, manganese, potassium, and zinc; and vitamin E. Three additional nutrients were significantly different in herbicide treated MON 88701 only (the amino acids methionine and proline, and the fatty acid 18:2 linoleic acid), while one additional nutrient was significantly different in untreated MON 88701 only (crude fiber) (Table VI-1, pages 107-122, and Table E-19, pages 426-439, in Monsanto 2012). No statistically significant differences were observed for 27 nutrients (Table VI-2, pages 123-131, and Table E-20, pages 440-448, in Monsanto 2012). With the exception of calcium (increased 14% and 15% relative to control in treated and untreated MON 88701 respectively), all differences in nutrient levels were 10% or less. In addition, most of the statistically significant differences in

nutrient levels were not consistently observed across locations, with the exception of calcium (statistically significant differences observed in seven of eight locations), ash (statistically significant differences observed at six (treated) and four (untreated) locations), 18:0 stearic acid (statistically significant differences observed at five locations), and manganese (statistically significant differences observed at five locations for untreated MON 88701 only). However, in all of these cases, the mean levels of all nutrients in MON 88701 were within the 99% tolerance interval established from the conventional commercial reference varieties. While the mean level of methionine in herbicide treated (but not untreated) MON 88701 were slightly outside of the 99% tolerance interval, the mean increase in methionine was less than 5%. Moreover, the increase was not consistently observed across locations (a statistically significant difference was observed at only one of the eight locations), and the mean level of methionine was within the natural variation observed for commercial cotton varieties (ILSI, 2010).

Statistically significant ($p < 0.05$) increases in combined-site anti-nutrient levels were observed for two anti-nutrients in both herbicide treated and untreated MON 88701: the cyclopropenoid dihydrosterculic acid and total gossypol. In addition, a statistically significant increase in free gossypol was observed in treated MON 88701. The increase was less than 10% in all cases except dihydrosterculic acid in untreated MON 88701, which increased 12.6%. Moreover, the increases were not consistently observed across locations and the anti-nutrient levels were all within the 99% tolerance interval established from the conventional commercial reference varieties. There were no statistically significant differences in the cyclopropenoids malvalic acid and sterculic acid (Tables VI-1, pages 107-122, VI-2, pages 123-131, E-19, pages 426-439, and E-20, pages 440-448, in Monsanto 2012).

The significant changes observed for the above-mentioned nutrients and anti-nutrients are unlikely to make MON 88701 more susceptible to pests and diseases, or to cause MON 88701 to have a greater impact on non-target organisms, than existing cotton varieties. As discussed further below, the disease, insect pest, arthropod abundance, and agronomic data presented for MON 88701 did not indicate any significant difference for the aforementioned observations.

Based on all the above noted data and considerations, APHIS concludes that MON 88701 poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional cotton varieties.

D. Potential Impacts on Disease and Pest Susceptibilities

USDA-APHIS assessed whether MON 88701 is likely to have significantly increased disease and pest susceptibility because of the introduced *dmo* and *bar* genes compared to the control cotton variety. This assessment encompasses a thorough consideration of the introduced traits, their impact on agronomic traits (discussed later in the document) and plant composition (discussed earlier), and quantitative and/or observational data on pest and disease responses. Important changes include those which would (1) affect not only

the new GE crop, but that would also result in significant introduction or spread of a damaging pest or disease to other plants; or (2) result in the introduction, spread, and/or creation of a new disease or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Important changes do not include changes in susceptibility to diseases and pests that are within the acceptable range of currently cultivated varieties.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, or weed programs exist (see http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml). Among these, PPQ's cotton pest programs specifically target boll weevil (*Anthonomus grandis*) and pink bollworm (*Pectinophora gossypiella*).

A number of other insects also feed on cotton. Between 2007 and 2011, the most important in terms of cotton yield loss were the bollworm/budworm (Heliothine) complex (*Helicoverpa zea* and *Heliothis virescens*), stink bugs (Pentatomidae), thrips (Thripidae), and *Lygus* species (Williams 2007, 2008, 2009, 2010, 2011). Of the various diseases affecting cotton, the most troublesome in recent years have been *Phymatotrichum* root rot (*P. omnivorum*), the boll rots, and the fungal seedling diseases (primarily *Rhizoctonia solani*, *Pythium spp*, and *Fusarium spp*) (Blasingame 2010; National Cotton Council 2011).

Cotton is not itself a plant pest in the U.S. (USDA-NRCS 2012a; 7 CFR part 340.2). The *A. tumefaciens* transformed plants used in the generation of MON 88701 were treated with antibiotics to kill *A. tumefaciens* cells (page 40 in Monsanto 2012). The description of the introduced genetic elements, expression of the gene products and their functions in MON 88701 has been summarized above. That inserted genetic material which was derived from plant pests does not result in the production of infectious agents or disease symptoms in plants.

Monsanto collected data relevant to cotton diseases and insect pests from field experiments conducted in two studies at a total of 15 (Study 1) and 11 (Study 2) locations across the United States during the 2010 growing season (Table VII-1, pages 138-140, and VII-4, page 151, in Monsanto 2012). These locations cover a diverse range of environmental conditions representative of most commercial cotton production areas and locations where MON 88701 is expected to be grown. All locations in Study 2 were also used in Study 1. The agronomic practices used to prepare and maintain each field site were characteristic of each respective region (page 533 in Monsanto 2012). In both studies, plant mapping data, disease and pest damage ratings, and arthropod abundance data were collected for control Coker 130 cotton and MON 88701. Data were also collected for 11 (Study 1) and eight (Study 2) commercial reference varieties, with four

varieties grown per site, to establish a range of natural variability for responses to the assessed stressors (Tables G-1 and G-2, pages 534-537 in Monsanto 2012).

Plant mapping is a process commonly used by agronomists and breeders to quantify cotton growth and development parameters such as boll retention (Kerby *et al* 2010). Final boll retention and distribution, as reflected in the plant mapping data, can provide an indication of the effect of abiotic and biotic on a cotton plant, as squares and early bolls tend to abort if the plant experiences stress (Guinn, 1982; Kerby *et al* 2010; University of California 1996). 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. Thus, if plant mapping results are similar between two cotton lines this generally indicates that the two lines had similar responses to the overall set of abiotic and biotic stresses experienced. In Study 2, plant mapping data was collected for MON 88701 treated with the herbicides glufosinate (0.5 lbs a.i./acre at the 3 – 5 leaf stage) and dicamba (0.5 lbs acid equivalent (a.e.)/acre at the 6-10 leaf stage) to allow for assessment of MON 88701 under the agronomic system in which it is expected to be used (pages 99 and 149 in Monsanto 2012). This data is important for this plant pest risk assessment since the DMO reaction product DCSA, which is formed in MON 88701 in the presence of dicamba, is an analog of salicylic acid. Salicylic acid and its endogenous derivatives are known to be involved in plant responses to stress, including to pests and pathogens, although their precise roles have not been fully elucidated (Bi *et al* 1997; Colson-Hanks *et al* 2000; Inbar *et al* 2001; Martinez *et al* 2000; Thaler *et al* 2010; Thaler *et al* 2012; Vlot *et al* 2009; Zarate *et al* 2007; Zhang *et al* 2011). It is plausible that DCSA could play a similar role as salicylic acid in plants or conversely, could interfere with salicylic acid mediated responses (Park *et al* 2009; Silverman *et al* 2005).

The researchers at each field site were expected to be familiar with the growth, production, and evaluation of cotton characteristics, and to use well-established qualitative and/or quantitative techniques to observe and evaluate environmental interactions. For plant responses to abiotic stress, disease damage and arthropod-related damage, at least three abiotic stressors, three diseases and three arthropod pests were evaluated four times at all sites, starting about 30 days after planting and continuing at approximately 30 days intervals. The researcher at each field site chose abiotic stressors, diseases and arthropod pests that were actively causing injury. When fewer than three stressors were present, the researcher chose additional stressors based on knowledge of those likely to occur in cotton during the given observation period. Therefore, the stressors typically varied between observations at a site or among sites, as did the number of observations for each stressor (Tables G-21 and G-22, pages 580-581, in Monsanto 2012). Qualitative damage ratings were collected from each plot using a continuous 0 – 9 scale of increasing severity and then grouped into four categories: none (0), slight (1-3), moderate (4-6), and severe (7-9). These qualitative categorical data were not statistically analyzed; they were considered different on a particular observation date at a site if the range of injury severity to MON 88701 did not overlap with the range of injury severity to the control across all four replications at each site (pages 540-541 in Monsanto 2012).

In addition to these qualitative assessments, in Study 1, quantitative damage assessments were performed at five sites for thrips (0 – 5 rating scale at 14, 21, and 28 days after planting) and heliothines (number of larvae and number of total and damaged fruiting bodies at 45, 60, 75, and 90 days after planting) (pages 540-541 in Monsanto 2012). At these same sites, arthropods were collected and quantified four times, starting about 30 days after planting and continuing at approximately 30 day intervals (page 542 in Monsanto, 2012). In both studies the total number of bolls per plant and the percent retention of first-position bolls were among the plant mapping characteristics assessed (Table VII-1, page 140, in Monsanto 2012). Quantitative numerical data for arthropod damage, arthropod abundance, and plant mapping characteristics underwent statistical analysis (page 543 in Monsanto 2012).

Neither the introduced traits nor dicamba and glufosinate herbicide treatment altered the assessed environmental interactions of MON 88701 compared to the conventional control. In Study 1, 498 out of 498 observations between untreated MON 88701 and the conventional control showed no meaningful differences in damage from 8 abiotic stressors, 14 diseases, and 14 types of arthropod pests (Tables VII-8, page 160, and G-20 – G-22, pages 579-581, in Monsanto 2012). Similarly, in Study 2, 385 out of 385 observations showed no meaningful differences in damage from these stressors (Tables VII-11, page 163, and G-27 – G-29, pages 591-593, in Monsanto 2012). Combined site quantitative analyses showed no difference between MON 88701 and the conventional control in damage caused by thrips or heliothine pests, or in the number of heliothine larvae in Study 1 (Tables VII-9 and VII-10, pages 160-161, in Monsanto 2012). The same results were seen at each individual site, except that there was less thrips and heliothine damage in MON 88701, and more live heliothine larvae, for a single time point at one site for each of these characteristics (Tables G-23 and G-25, pages 582-583, in Monsanto 2012). There were also no differences seen in Study 1 in pest abundance between MON 88701 and the conventional control for 87 out of 89 observations across five sites at four time points for 10 different pests (page 161 and Table G-25, pages 584-587 in Monsanto 2012). Fewer stinkbugs and tarnished plant bugs were observed in MON 88701 at one site and one time point were observed in Study 1 for 87 out of 89 observations.

No differences were observed in combined-site analysis of 6 plant mapping characteristics, including the total number of bolls per plant and the percent retention of first position bolls, between MON 88701 and conventional control. There was a small but statistically significant increase in the average number of first position bolls in MON 88701 relative to control. However, this value was within the reference range established using eight reference varieties and would not indicate an increase susceptibility of MON 88701 to stress (Table VII-7, page 157, in Monsanto 2012). The same results were seen when MON 88701 was treated with dicamba and glufosinate (Table G-18, page 576, in Monsanto, 2012), indicating that herbicide treatment did not significantly affect the overall response of MON 88701 to the set of abiotic and biotic stresses experienced during the growing season.

Finally, as discussed earlier, there were no significant changes in MON 88701 compositions that would render MON 88701 more susceptible to pests and diseases over its control or reference cotton varieties. As presented later in this document, the observed agronomic traits also did not reveal any significant changes that would indirectly indicate that MON 88701 is or could be relatively more susceptible to pests and diseases over control or reference cotton varieties. Thus MON 88701 is expected to be susceptible to the same plant pathogens and insect pests as conventional cotton. The introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on MON 88701 over the conventional control. For this reason, there is also unlikely to be any indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

MON 88701 is not engineered for pest resistance, thus there are no ‘target’ species, and thus no ‘nontarget’ species either. However, APHIS assessed whether exposure or consumption of herbicide-resistant MON 88701 containing the DMO and PAT proteins would have an adverse effect on beneficial species or wildlife associated with cotton.

Monsanto provided the following information justifying the safety of MON 88701 (Sections V.D-V.F, pages 88-97, in Monsanto 2012):

- The donor organisms, *S. maltophilia* and *S. hygroscopicus*, are not known for human or animal pathogenicity. *S. maltophilia* is an aerobic, ubiquitous, environmental, gram-negative bacterium which can be found in healthy individuals without causing harm to human health. Its incidental presence on foods and crops without any adverse reports establishes the safety of the donor organism (page 90 in Monsanto 2012). It has been previously reviewed as part of safety and nutritional assessments of other genetically engineered crop products (US-FDA 2011). *S. hygroscopicus* is a saprophytic, gram-positive, 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) (pages 92-93 in Monsanto 2012).
- The DMO enzyme present in MON 88701 has sequence similarity and many catalytic and domain structural similarities with a wide variety of oxygenases found in numerous species of microorganisms widely distributed and prevalent in the environment (Chakraborty *et al* 2012). It also has similarity with oxygenases such as pheophorbide A oxygenase which are found in plants such as rice, maize, canola and pea (Rodoni *et al* 1997; Yang *et al* 2004) that are consumed in a variety of food and feed sources which have a history of safe human consumption. Plants, animals and humans are extensively exposed to these types of enzymes (pages 90-91 in Monsanto, 2012).
- The PAT enzyme present 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. OECD recognizes PAT proteins produced from

different genes to be equivalent with regard to function and safety (OECD 1999). PAT proteins are structurally similar only to other acetyltransferases known to not cause adverse effects after consumption (Herouet *et al* 2005). In 1997, a tolerance exemption was issued for PAT proteins by the U.S. EPA (U.S. EPA 1997).

- Bioinformatics analyses demonstrated that the DMO and PAT proteins in MON 88701 do not share amino acid sequence similarities with known allergens, gliadins, glutenins, or proteins toxins which could have adverse effects to human or animal health (pages 88 – 89 in Monsanto 2012).
- The DMO and PAT proteins in MON 88701 are readily digestible in simulated gastric and intestinal fluids, making it highly unlikely that it would be absorbed in the small intestine and have any adverse effects on human or animal health (pages 94-95 in Monsanto 2012).
- Acute oral toxicity studies have indicated that the DMO and PAT proteins have no adverse effects in mice at the highest dose tested (Herouet *et al* 2005; page 84 in Monsanto 2010). By extrapolation, there is no meaningful risk to human or animal health from dietary exposure to MON 88701.

As indicated earlier in this plant pest risk assessment, the petitioner's characterization of MON 88701 showed that it is similar in nutritional and compositional analysis to the unmodified control variety Coker 130 (Section C. Expression of the gene product, enzymes or changes to plant metabolism). Gossypol and the cyclopropenoid sterculic acid play a role in defense of cotton against insect pests (Chan et al 1978; Kong et al 2010; Rani and Rajasekharreddy 2012), suggesting that increases in these compounds could have negative effects on non-target organisms. Although statistically significant increases in combined-site levels of gossypol and the related cyclopropenoid fatty acid dihydrosterculic acid were observed in both herbicide treated and untreated MON 88701, the increases were not consistently observed across locations and the levels of these compounds were all within the 99% tolerance interval established from the conventional commercial reference varieties (page 105 and Tables VI-1, pages 109-110, and E-19, page 428, in Monsanto 2012).

As discussed in the previous section, Monsanto conducted field experiments in two studies at locations across the United States during the 2010 growing season. In one of these studies, arthropods were collected and quantified four times at five locations, starting about 30 days after planting and continuing at approximately 30 day intervals (page 542 in Monsanto 2012). No differences were seen in the abundance of seven different beneficial arthropods between MON 88701 and the conventional control for 86 out of 89 observations (page 161 and Table G-26, pages 588 – 590, in Monsanto 2012). More damselbugs were seen at one location for one of four time points, while fewer *Orius* species (which include predators of thrips) were observed at one location for two of three time points. Although the mean abundance values for *Orius* species at this site were outside the reference range established by four commercial cotton varieties also grown at the site, differences in *Orius* abundance were not consistently detected across sites.

Based on APHIS's analyses of the data provided by Monsanto (as described here and in section C. Expression of the gene product, enzymes or changes to plant metabolism)

MON 88701 is unlikely to cause any significant adverse effects on nontarget organisms (including beneficial species or wildlife associated with cotton) compared to other commercial cotton varieties. Any effects on non-target organisms that could potentially result from proposed changes in herbicide labels will be evaluated by the U.S. EPA. APHIS has concluded that adverse impacts to non-target organisms exposed to MON 88701 are unlikely.

F. Potential for Enhanced Weediness of MON 88701

Upland cotton (*G. hirsutum*) possesses few of the characteristics common to plants that are successful weeds (Baker 1965; Keeler 1989) and is not considered to be a serious or common weed in the United States. It is not listed as a weed in the major weed references (Crocket 1977; Holm *et al* 1979; Muenscher 1980), nor is it present on Federal or State lists of noxious weed species (USDA-APHIS 2012; USDA-NRCS 2012b). Modern Upland cotton is a domesticated perennial grown as an annual crop that is not generally persistent in unmanaged or undisturbed environments without human intervention. Modern cultivars are not frost tolerant and do not survive freezing winter conditions, do not produce abundant or long-lived seeds that can persist or lie dormant in soil, do not exhibit vegetative propagation or rapid vegetative growth, and do not compete effectively with other cultivated plants (OECD 2008). In areas where winter temperatures are mild and freezing does not occur, cotton plants can occur as volunteers in the following growing season. However, these volunteers can be easily controlled by herbicides or mechanical means. Cotton can become locally feral or naturalized in suitable areas, such as southern Florida, Hawaii, and Puerto Rico (Coile and Garland 2003; Fyxe 1979; USDA-NRCS 2012a; Wunderlin and Hansen 2008).

Monsanto collected seed germination data (as an indicator of seed dormancy) for seed grown at three field locations and tested under controlled laboratory conditions at six different temperature regimes (10 °C, 20 °C, 30°C and three diurnal combinations of these temperatures). Germination data were collected for MON 88701 seed, control Coker 130 seed, and nine commercial reference varieties (pages 145-147 in Monsanto 2012). In a combined-site analysis, no statistically significant differences ($p < 0.05$) in seed germination rates, dead seed, or viable but ungerminated seed were observed for five of the temperature regimes. At 30 °C, MON 88701 seed germinated at a slightly higher rate than Coker 130 seed (96.7% versus 94.4%) and had a correspondingly lower amount of dead seed. However, these slight differences were well within the range of rates observed for the commercial reference varieties. Moreover, at no temperature regime was viable hard seed observed. Therefore, APHIS concludes that there were not biologically meaningful differences in the seed germination characteristics between MON 88701 and its parental control.

Monsanto also collected agronomic data relevant to weedy traits such as plant vigor and height and seed yield from field experiments conducted in two studies at a total of 15 (Study 1) and 11 (Study 2) locations across the United States during the 2010 growing season (Tables VII-1, pages 138-40, and VII-4, page 151, in Monsanto 2012). All locations in Study 2 were also included in Study 1. Data were collected for control Coker 130 cotton and MON 88701, as well as for MON 88701 treated with the herbicides

glufosinate (0.5 lbs a.i./acre at the 3 – 5 leaf stage) and dicamba (0.5 lbs a.e./acre at the 6-10 leaf stage) to allow for assessment of MON 88701 under the agronomic system that it is expected to be used (pages 99 and 149 in Monsanto 2012). Data were also collected for 11 (Study 1) and eight (Study 2) commercial reference varieties (four varieties grown per site) to establish statistical tolerance intervals for the various traits assessed (Tables G-1 and G-2, pages 534-537 in Monsanto 2012).

Plant vigor was assessed qualitatively. No differences in vigor were observed between MON 88701 and the Coker 130 control at 14 and 30 days after planting for 73 out of 74 comparisons across all sites and treatments. At one site, MON 88701 plants were slightly less vigorous than Coker 130 at 30 days after planting, but were within the range of vigor ratings of the commercial reference varieties.

Other agronomic data were assessed quantitatively. In the combined-site analysis, no statistically significant ($p < 0.05$) differences were observed between MON 88701 and the Coker 130 control for stand count at 14 and 30 days after planting, final stand count, seedcotton yield, number of immature seed per boll, boll weight, or a variety of fiber characteristics. In contrast, in both studies and whether untreated or herbicide treated, MON 88701 plants were shorter than Coker 130 control plants, took slightly longer to mature (as indicated by an increased number of nodes above white flower), produced more but smaller seed, and had a slightly increased fiber strength (Tables VII-5, page 153, VII-6, page 155, and G-13, page 567, in Monsanto 2012). These differences were all small and in all cases the mean values for these characteristics were within the range observed for the commercial reference varieties. Finally, as discussed above, changes in disease or insect pest susceptibility or in response to abiotic stress were not observed in MON 88701 relative to control.

Given these data, the herbicide-resistance traits conferred by the *dmo* and *bar* genes are very unlikely to provide MON 88701 with a selective advantage in unmanaged ecosystems. However, the herbicide-resistance traits could complicate efforts to control volunteer cotton in settings where dicamba and/or glufosinate are being applied for weed control, such as in subsequent cotton (planted on over 50% of cotton acreage grown in 2010, see Table VIII-20, page 217 in Monsanto 2012) or rotation crops (Roberts *et al* 2002; Fannin 2010; Ledbetter 2011). In particular, dicamba was used for weed control on 20% of sorghum acres in 2011 (sorghum was planted as a rotation crop on 8% of cotton acreage in 2010) and in 11% of winter wheat acres in 2009 (wheat was planted as a rotation crop on 9% of cotton acreage in 2010), as well as in 7% of cotton acres in 2007 (Table VIII-20, page 217, in Monsanto 2012). Although cotton volunteers typically do not reduce crop yield, they can act as reservoirs for insect pests of cotton (York *et al* 2004). However, both mechanical means (tillage) and a variety of other herbicide treatments are available for control of volunteer cotton in such circumstances (Keeling *et al* 2009; Morgan *et al* 2011; Thompson and Steckel 2009; Fannin 2010; Ledbetter 2011; York *et al* 2004; see also pages 228-230, including Table VIII-25, in Monsanto 2012). For instance, volunteer MON 88701 in subsequent corn (planted as a rotation crop on 16% of cotton acreage, Table VIII-20, page 217, in Monsanto 2012), sorghum, or wheat crops can be well controlled at various time points with the herbicides Sharpen, atrazine,

2,4-D, Status (a mix of diflufenzopyr and dicamba), or Starane (Morgan *et al* 2011; Fannin 2010; Table VII-25, page 230, in Monsanto 2012). The specificity of the DMO and PAT proteins for dicamba and glufosinate respectively is high and other common herbicides are not metabolized by these enzymes (see Section C above). Therefore, excepting dicamba and glufosinate, MON 88701 is expected to be sensitive to the same herbicides as other cotton varieties.

Therefore, based on this characterization, APHIS concludes that MON 88701 is no more likely to establish weedy populations than existing cotton cultivars, and such populations can be controlled using current weed control practices.

G. Potential Impacts on the Weediness of Any Other Plants with which It Can Interbreed

Gene introgression is the movement of a gene or genes from one population of organisms into the gene pool of another, genetically distinct, population via sexual crossing. The process begins with the pollination of one species by the other, followed by the establishment of one or more hybrid offspring and maintenance of introgressed genes during repeated backcrossing of the hybrid to one of the parental species or to a different species. Gene introgression is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Hegde *et al.* 2006; Rieseberg 1997; Soltis and Soltis 1993), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Peterson *et al* 2002; Rieseberg and Wendel 1993; Stace 1987). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars. However, gene introgression from crops to wild relatives is also thought to have a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and few other crops (see Table 1 in Ellstrand *et al* 1999).

APHIS considers two primary issues when assessing weediness of sexually compatible plants because of transgene flow: 1) the potential for transgene flow and introgression to sexually compatible cultivated, wild, or free-living relatives and, 2) if transgene flow and introgression are determined to be biologically meaningful, the potential impact of introgression with respect to weediness of the recipient.

Potential for hybridization and gene introgression

Two cultivated and two wild species of cotton grow in the United States and its territories. *G. hirsutum* (Upland cotton) is the most widely cultivated species, comprising 97% of the U.S. cotton crop (USDA-NASS 2012a). The vast majority of Upland cotton is cultivated in the Cotton Belt, which stretches across the southern United States from Virginia to California (USDA-NASS 2012a). Small amounts are also grown in Puerto Rico for breeding and seed production purposes (Bayer CropScience 2006; Monsanto

Company 2004). In addition to cultivated varieties, naturalized or native³ populations of *G. hirsutum* grow in Florida, Puerto Rico, and the Virgin Islands while naturalized populations grow in some of the Hawaiian Islands (Coile and Garland 2003; Fryxell 1984; USDA-NRCS 2012a; Wunderlin and Hansen 2008). The second cultivated species, *G. barbadense* (Pima or Egyptian cotton), is grown in Arizona, California, New Mexico, and Texas (Pleasants and Wendel 2005; USDA-NASS 2012a). Naturalized populations of *G. barbadense* grow in Puerto Rico, the Virgin Islands and most of the major Hawaiian Islands (Bates 1990; Fryxell 1984; USDA-NRCS 2012d). Two wild species of cotton are native to the United States, *G. thurberi* and *G. tomentosum*, and grow in Arizona and Hawaii respectively (Fryxell 1984; USDA-NRCS 2012e; USDA-NRCS 2012f).

G. hirsutum is tetraploid and thus effectively incompatible with diploid species such as *G. thurberi*. Plants from these two groups do not normally hybridize spontaneously and produce fertile offspring, and experimental crosses are difficult (OECD 2008). In contrast, *G. hirsutum* is sexually compatible with the tetraploids *G. barbadense* and *G. tomentosum* and can form viable and fertile progeny with both species (Brubaker *et al* 1993; OECD 2008; Saha *et al* 2006). Thus, unassisted outcrossing and gene introgression could potentially occur in areas where these species are co-located.

Wind dispersal of cotton pollen is negligible because of its large size and self-adherent properties (Hutmacher *et al* 2006; Jenkins 1993; OECD 2008). However, cross-pollination between cotton species can occur through the activity of pollinating insects (Llewellyn *et al* 2007; McGregor 1976; OECD 2008; Van Deynze *et al* 2005). For transgene introgression from MON 88701 to occur there would have to be spatial proximity between MON 88701 and the recipient variety or species, overlap in their flowering phenology, and overlap in their pollinators (Pleasants and Wendel 2005). In general, the extent of transgene introgression will depend on the species pool, preferences, and abundance of pollinators, which can vary according to region, location, season, time of day, and use of insecticides. In addition, transgene introgression will decrease with increasing geographic distance between the source and receiver populations and physical barriers and intermediate pollinator-attractive plants can reduce the potential for pollen movement (Green and Jones 1953; Llewellyn *et al* 2007; McGregor 1976; OECD 2008; Umbeck *et al* 1991; Van Deynze *et al* 2005; Zhang *et al* 2005). Additional information on the biology of cotton can be found within the OECD cotton consensus document (OECD 2008).

Although naturalized populations of *G. hirsutum* grow in Southern and Central Florida, their northernmost reported location (Gilchrest County, FL)) is separated by over 120 miles from the nearest commercial cotton production areas in the Florida panhandle (Wunderlin and Hansen 2008; USDA-NASS 2012b). Thus, outcrossing from MON 88701 to naturalized *G. hirsutum* in Florida is highly unlikely.

³ A “native” plant is one that has grown in a particular region or ecosystem for hundreds or thousands of years without intentional or accidental human help. A “naturalized” plant is a non-native plant that does not need human help to reproduce and maintain itself over time in an area where it is not native. (USDA-NRCS 2012c).

In contrast, *G. hirsutum* is cultivated in many areas where *G. barbadense* is also grown (USDA-NASS 2012b). In addition, as noted above, native and/or naturalized populations of both species are present in Hawaii, Puerto Rico, and the Virgin Islands. Although cultivated varieties of both species are largely self-pollinated, insect-mediated cross-pollination can occur both within and between the species (Brubaker *et al* 1993; Llewellyn *et al* 2007; OECD 2008; Van Deynze *et al* 2005; Van Deynze *et al* 2011). Bumble bees (*Bombus* spp.), *Melissodes* and *Halictus* bees, honey bees (*Apis mellifera*), and *Scolia* wasps are the primary pollinators (McGregor 1976).

Published studies report that there has been relatively little gene introgression from *G. hirsutum* into native or naturalized *G. barbadense* in Mesoamerica and the Caribbean, despite the fact that *G. barbadense* has been grown in the presence of the predominant *G. hirsutum* since prehistoric times. In contrast, introgression from *G. barbadense* to native or naturalized *G. hirsutum* in these areas has been relatively common (Brubaker *et al* 1993; Wendel *et al* 1992). Various mechanisms have been suggested to account for this asymmetry (Brubaker *et al* 1993; Jiang *et al* 2000; OGTR 2008; Percy and Wendel 1990). While none of these mechanisms leads to complete isolation between the two species, the reported asymmetry in gene flow suggests that gene introgression from cultivated *G. hirsutum* varieties such as MON 88701 to native or naturalized *G. barbadense* should be rare.

However, gene introgression from cultivated *G. hirsutum* to cultivated *G. barbadense* may be more likely, since gene flow between cultivated varieties of these species appears to occur with the opposite asymmetry from that observed between native or naturalized varieties (CDFA 2009; Van Deynze *et al* 2011). The mechanism underlying this reversal in the directionality of gene flow, which has also been reported in Caribbean accessions (Wendel, Brubaker *et al*. 1992; Brubaker, Jason *et al*. 1993), is not known. Nonetheless, outcrossing rates from MON 88701 to cultivated *G. barbadense* are still likely to be low. For instance, Van Deynze *et al*. (2005) reported that Upland/Pima hybrid plants have been observed at a rate of 0.01% in fields sown with seeds of cultivated varieties that were obtained from production fields separated by at least 800 meters.

With regard to *G. tomentosum*, natural populations of this species are found on all Hawaiian Islands except Kauai and Hawaii; the species is dominant on Kohoolawe and several sizable populations are found on Oahu and Maui. Populations are located on the drier, leeward coastal plains of the islands at low elevations, which are also the areas that are primarily used for agriculture (Pleasants and Wendel 2005). *G. hirsutum* has not been grown as an agricultural commodity in Hawaii for decades, and to the best of APHIS' knowledge, seed companies no longer use the Hawaiian Islands as a winter nursery (Grace, personal communication, 2012).

The flowering period for *G. tomentosum* corresponds to the end of the rainy season; it may begin as early as January, with peak flowering occurring in April and May, and may extend through August in a very wet year (Pleasants and Wendel 2010). Thus, any cultivated cotton that blooms between January and August could potentially overlap with *G. tomentosum*. Previously, it was thought that peak anthesis and receptivity in *G.*

tomentosum occurs at dusk, whereas in *G. hirsutum* the flowers open in the morning and wither by evening (OECD 2008). However, Pleasants and Wendel (2010) found that *G. tomentosum* flowers also open in the morning, dehisce rapidly, and begin to senesce by late afternoon. These results suggest that there is substantial overlap in flowering phenology between *G. hirsutum* and *G. tomentosum*.

Spontaneous self-pollination is rare in *G. tomentosum*, perhaps due to the structure of its flowers. Instead, the species appears to rely on the action of pollinators (Münster and Wiczorek 2007; Pleasants and Wendel 2005). It was previously thought that moths were the only insects that pollinated *G. tomentosum*, and thus that there was little overlap with pollinators of *G. hirsutum* (OECD 2008; Pleasants and Wendel 2005). However, more recent studies have shown that *G. tomentosum* is pollinated by honeybees and carpenter bees, which are among the species that also pollinate commercially grown *G. hirsutum*. In addition, both of these pollinators are long-distance foragers; for instance, honeybees may forage up to 6 – 10 miles from their nest (Pleasants and Wendel 2010). Thus, in addition to overlap in flowering phenology, there is overlap in pollinators between *G. tomentosum* and *G. hirsutum*. However, no hybrids between *G. hirsutum* and *G. tomentosum* have been identified to date, although only a relatively small number of accessions and marker loci have been examined (DeJode and Wendel 1992).

Expression of the DMO and PAT proteins does not cause any major changes in the phenotype of cotton plants other than to confer resistance to the herbicides dicamba and glufosinate. No significant differences in viability, size, or morphology were observed in pollen obtained from MON 88701 compared to pollen from the conventional control (pages. 164-166 in Monsanto 2012). Thus, the introduced genetic material is unlikely to cause an increased rate of outcrossing of MON 88701 relative to non-transgenic varieties. Should outcrossing from MON 88701 to *G. barbadense* or *G. tomentosum* occur, transgene introgression would still require the establishment of hybrid progeny followed by persistence of the transgene through self-crossing or back-crossing into the recipient species in subsequent generations.

The low level of introgression from *G. hirsutum* to native or naturalized *G. barbadense* observed in the Caribbean and the phenomenon of hybrid breakdown suggests that transgene introgression from MON 88701 to native or naturalized *G. barbadense* can occur but is likely to be rare (Jiang *et al* 2000; OGTR 2008). In the absence of herbicide treatment, the transgenic material in MON 88701 is unlikely to confer a selective advantage on any hybrid progeny that may result from outcrossing. Thus, the transgenes present in MON 88701 are unlikely to increase the rate of successful transgene introgression from MON 88701 into native or naturalized *G. barbadense* relative to the rate of gene introgression from conventional cultivars.

Transgene introgression from MON 88701 to cultivated *G. barbadense* can also occur but is also likely to be rare since cultivated *G. barbadense* is regularly harvested. While the likelihood of transgene movements to *G. barbadense* is likely greater with cultivated varieties than with native or naturalized *G. barbadense*, such movements would tend to involve plants producing seeds intended for processing rather than planting because seed

production fields are isolated from commercial fields. Seed production isolation standards will help ensure that any movement of transgenes into seed production fields will remain at very low levels (AOSCA 2003; Van Deynze *et al* 2005). The transgenes present in MON 88701 unlikely to increase the rate of successful transgene introgression from MON 88701 into cultivated *G. barbadense* relative to the rate of gene introgression from conventional cultivars.

Finally, introgression into *G. tomentosum* in Hawaii is also likely to be rare, both because of barriers to introgression (Brubaker *et al* 1993; Jiang *et al* 2000; OGTR 2008; Percy and Wendel 1990), and because there is no commercial cotton production on these islands (Grace, personal communication, 2012). If any Upland cotton is grown in the Hawaiian Islands, it is grown at a very small scale and outcrossing to *G. tomentosum* is unlikely to occur. Should outcrossing nonetheless occur, transfer of the transgenes present in MON 88701 would not be expected to confer a selective advantage on the hybrid progeny or to reduce hybrid breakdown, which would be expected to eliminate introgressed genes from the *G. tomentosum* population. Thus, the transgenes present in MON 88701 are unlikely to increase the rate of successful transgene introgression from MON 88701 to *G. tomentosum*.

In summary the available evidence indicates that there is a low potential for introgression of transgenic material from MON 88701 cotton to *G. tomentosum* or to native or naturalized *G. barbadense*. There is no evidence that any of the genetic elements used in MON 88701 would increase the rate of outcrossing or gene introgression of MON 88701 relative to non-transformed cotton.

Relative fitness/weediness of recipients after introgression

As discussed in the previous section, the genetic material introduced into MON 88701 does not confer or enhance weedy characteristics of cultivated Upland cotton. There is no reason to believe that it would do so in naturalized or native *G. hirsutum*, in *G. tomentosum*, or in cultivated, naturalized, or native *G. barbadense*. Thus, in the unlikely event that transgene introgression from MON 88701 to one of these other types of cotton were to occur, the herbicide resistance traits would provide a selective advantage only when the resulting hybrids were in contact with the herbicide (i.e., in an agricultural field or treated rights of way). However, APHIS could find no reports that any of these potential recipient populations are actively controlled by herbicides. Therefore, transgene introgression from MON 88701 would not be expected to adversely impact recipient plants or increase their fitness or weediness any more than would gene flow from cultivated non-transgenic Upland cotton. Nor would it affect efforts to remove wild populations, as no such efforts exist.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS considered whether there are likely to be significant changes to agricultural practices associated with cultivation of MON 88701, and if so, are they likely to significantly exacerbate plant diseases or pests, especially those for which APHIS has a control program. Relative to currently cultivated cotton varieties, the only agricultural or

cultivation practices that are expected to change if MON 88701 is no longer subject to regulation are those related to weed management: in particular, choice of herbicide(s) or herbicide combinations, times of application.

The current and proposed uses of dicamba in cotton are described in the petition (Section VIII.G, pages 206- 214, in Monsanto 2012). Dicamba is currently labeled only for early preplant applications in cotton. In addition, due to insufficient crop tolerance of cotton to applications of dicamba, preplant restrictions are required to avoid cotton injury: a maximum application rate of 0.25 lbs a.e. per acre and a minimum of 21 days and one inch of rainfall or overhead irrigation between preplant application and planting of cotton. Due to insufficient crop tolerance, dicamba currently also cannot be used for in-crop postemergence applications. If EPA approves Monsanto's submitted application to amend Registration Number 524-582 for a DGA salt formulation of dicamba, growers would be authorized to apply dicamba alone or in mixtures with glufosinate, glyphosate (when MON 88701 is stacked with glyphosate resistant cotton) or other herbicides for preplant applications without restrictions and for in-crop postemergence applications on MON 88701. Non-aerial applications of dicamba would be authorized preemergence up to crop emergence as a single application or split applications up to a total of 1.0 lb a.e. per acre, while up to two postemergence applications of up to 0.5 lb a.e. per acre each through seven days prior to harvest. The total maximum annual application rate would be 2.0 lb dicamba a.e. per acre. The use of dicamba on MON 88701 is not expected to impact dicamba-sensitive crops from drift (spray or volatility) because Monsanto is registering a low volatility (DGA salt) dicamba formulation for use on MON 88701 and aerial applications of dicamba would not be allowed. Issues related to herbicide drift and volatilization will be further addressed in the NEPA document for this petition.

Upon integration of MON 88701 into glyphosate resistant cotton systems, aside from the anticipated dicamba label changes requested, Monsanto expects that growers will have the ability to continue to use established cotton production practices including the crop rotation, tillage systems, labeled herbicides, and planting and harvesting machinery currently being utilized (Section VIII.G, pages 206 – 212, in Monsanto 2012). The anticipated label changes would facilitate a wider window of application for dicamba in cotton, which is expected to provide a tool for improved control of broadleaf weeds (including some with resistance to other herbicides such as glyphosate, ALS, and PPO chemistries) that can be integrated into weed management programs using no-till or reduced tillage or conventional tillage. Monsanto's anticipated weed management recommendations for MON 88701 combined with glyphosate resistant cotton also include a preemergence (burndown at planting) application of a residual herbicide either alone or combined with glyphosate and dicamba depending on location and the type of tillage employed (Tables VIII-16 and VIII-17, pages 208-209, in Monsanto 2012). The impacts of this system for reducing or managing weeds and the evolution of herbicide-resistant weeds will be examined as part of the NEPA analysis. Greater weed control could potentially reduce disease and pest pressure in cotton if the diseases and pests of the weeds also use cotton as a host.

Crop rotation practices are not expected to be adversely impacted by the use of dicamba on fields planted to MON 88701. Crop rotation practices in cotton were analyzed in the petition (Section VIII.H, pages 215-230, in Monsanto 2012). The primary crops planted after cotton are cotton (54%), corn (16%), wheat (9%), soybean (8%), sorghum (8%), and peanut (4%). Dicamba can be absorbed through leaves and roots and translocated, but is considered only moderately persistent in soil, with a half-life of six days for dicamba acid under aerobic soil conditions with formation of the non-persistent degradate DCSA, and a half-life of 141 days under anaerobic soil conditions (U.S. EPA 2009). Crop rotation restrictions range from 30 to approximately 180 days, depending on the rate applied, inches of rainfall and the following crop, according to the Clarity® label (BASF 2010), and these should be adequate for rotation to other crops the spring following harvest of cotton.

As described above (see *Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities*), field studies on MON 87701 cotton demonstrated that neither the herbicide resistance traits nor the herbicide treatments appear to alter the response of MON 87701 cotton to abiotic stress, diseases, or arthropod pests under natural levels of these stressors, nor were pest arthropods more abundant around MON 88701 plots. Agronomic practices used to prepare and maintain each study site were characteristic of those used in each respective geographic region and all maintenance operations were performed uniformly over the entire trial area (page 533 in Monsanto 2012). Although pest and disease susceptibility data was not presented for MON 88701 stacked with the glyphosate-resistant trait, a recent review indicates that neither the glyphosate resistance trait nor glyphosate use in glyphosate resistant crops increases crop disease (Duke *et al* 2012), and there is no evidence that either increase susceptibility to insect pests. Therefore, changes in agricultural practices related to weed control in MON 88701 or MON 88701 stacked with the glyphosate resistance trait are unlikely to adversely impact pest and disease control practices or any other cultivation and management practices in cotton.

In conclusion, as discussed throughout this document, MON 88701 is similar to conventional cotton in its agronomic, phenotypic, environmental, and compositional characteristics and has levels of tolerance to insects and diseases comparable to conventional cotton. Therefore, no significant adverse impacts on current agricultural or cultivation practices are expected following the introduction of MON 88701.

I. Potential Impacts from Transfer of Genetic Information to Organism with which MON 87701 Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 88701 to be horizontally transferred without sexual reproduction to other organisms (horizontal gene transfer (HGT)) and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests or pathogens. HGT between unrelated organisms has been intensively studied in recent years, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge *et al* 1998). Potential risks from HGT from genetically

engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes and viruses and in the long run has contributed to major transitions in evolution.

Potential for horizontal gene transfer to bacteria or fungi

MON 88701 has two bacterial genes. HGT and expression of DNA from a plant species to other bacterial species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), there are almost no evolutionary examples of HGT to bacteria from eukaryotes or from plants to fungi (Keese 2008). The only genes likely to be transferred successfully from genetically engineered plants to bacteria are other bacterial genes. Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of MON 88701 is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants, including *Agrobacterium* and *Rhizobium* (Kaneko *et al* 2002; Wood *et al* 2001), and there is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data has implied that HGT has occurred, these events are inferred to have occurred on an evolutionary time scale on the order of millions of years (Brown 2003; Koonin *et al* 2001). Third, under natural conditions; no transfer of an intact functional gene from a genetically engineered plant to bacteria or fungi has been demonstrated to date (Miki and McHugh 2004). Fourth, the transgene DNA promoters and coding sequences used in MON 88701 are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Finally, the FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (U.S. FDA 1998). Therefore, APHIS concludes that horizontal gene transfer from MON 88701 to bacteria or fungi is unlikely to occur and thus poses no significant environmental or plant pest risk.

Potential for horizontal gene transfer to viruses

APHIS also considered whether horizontal transfer of DNA from MON 88701 to plant viruses was likely to occur and would lead to the creation or selection of a more virulent plant pathogen through recombination with other plant viruses. This issue has been considered before by other science review panels and government regulatory bodies (Keese 2008). The only virus sequences contained within MON 88701 encode regulatory elements from the peanut chlorotic streak virus, the tobacco etch virus, and the cauliflower mosaic virus. Regulatory elements such as promoters and terminators have

not been implicated in viral recombination. Therefore, APHIS concludes that horizontal transfer of DNA from MON 88701 to viruses is unlikely to occur and thus poses no significant environmental or plant pest risk.

Potential for horizontal gene transfer to parasitic plants

Recently, Yoshida *et al* (2010) through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*), which infests cereal fields (monocots) including corn and sorghum (*Sorghum bicolor*). According to this study, incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this horizontal gene transfer occurred before the speciation of purple witchweed (*S. hermonthica*) and related cowpea witchweed (*S. gesnerioides*), a parasitic plant of dicots, from their common ancestor. In other words, HGT between a parasitic plant and its host is an extremely rare event. Therefore, APHIS concludes that horizontal gene transfer from MON 88701 to parasitic plants is unlikely to occur and thus poses no significant environmental or plant pest risk.

J. Conclusion

APHIS prepared this plant pest risk assessment in order to determine if MON 88701 is unlikely to pose a plant pest risk. The inserted genetic material does not pose a plant pest risk. MON 88701 is no weedier than the unmodified control or commercial varieties. There is low potential for introgression of transgenic material from MON 88701 cotton to sexually compatible wild or naturalized relatives, and should such introgression occur it would not be expected to increase the fitness or weediness of the recipient plants any more than would gene flow from cultivated non-transgenic Upland cotton. MON 88701 also exhibits no greater susceptibility to disease or plant pests than the unmodified control or commercial varieties, is unlikely to cause deleterious effects on non-target or beneficial organisms in the agro-ecosystem, and is unlikely to adversely affect agricultural or cultivation practices. Horizontal gene transfer of the introduced material to non-sexually compatible organisms is extremely unlikely to occur. Therefore, APHIS has concluded that MON 88701 is unlikely to pose a plant pest risk.

K. References

7 Code of Federal Regulations, Part 340 [1-1-12 Edition]. Introduction of organisms and products altered or produced through genetic engineering which are plant pests or which there is reason to believe are plant pests. Retrieved on August 4, 2012 from <http://www.gpo.gov/fdsys/pkg/CFR-2012-title7-vol5/pdf/CFR-2012-title7-vol5-part340.pdf>

7 United States Code, Sections 7701-7772 [2011 Edition]. Plant Protection Act. Retrieved on August 4, 2012 from <http://www.gpo.gov/fdsys/pkg/USCODE-2011-title7/pdf/USCODE-2011-title7-chap104.pdf>

40 Code of Federal Regulations, Part 180.227 [7-1-11 Edition]. Dicamba; tolerances for residues. Retrieved on July 20, 2012 from <http://www.gpo.gov/fdsys/pkg/CFR-2011-title40-vol24/pdf/CFR-2011-title40-vol24-sec180-227.pdf>

51 Federal Register 23302 [June 26, 1986]. Coordinated Framework for Regulation of Biotechnology. Retrieved on August 9, 2012 from <http://www1.usgs.gov/usbiotechreg/index.jsp>

Adrian-Romero M, G Blunden, BG Carpenter and E Tyihak. 1999. HPLC quantification of formaldehyde, as formal demethone, in plants and plant-like organisms. *Chromatographia* 50(3/4):160-166.

AOSCA. 2003. "Yellow Books" 2003: Operational Procedures, Crop Standards and Service Programs Publication (Genetic and Crop Standards). Association of Official Seed Certifying Agencies Meridian, ID.

Awmack CS and SR Leather. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47:817-844.

Baker HG. 1965. Characteristics and modes of origin of weeds. pp. 147-168. *In* HG Baker and GL Stebbins (eds), *The Genetics of Colonizing Species*. London: Academic Press.

BASF. 2010. CLARITY® Herbicide Label, Soybean, pp. 18-19. BASF Corporation 007969-00137.20100927c.NVA 2010-04-065-0154, 22 pp. Retrieved on June 27, 2012 from <http://agproducts.basf.us/app/cdms?manuf=16&pd=229&ms=2274>

Bates, DM. 1990. Malvaceae. *Manual of the flowering plants of Hawaii*. WL Wagner, DR Herbst and SH Sohmer. Honolulu, University of Hawaii Press, Bishop Museum Press: 868-902.

Bayer CropScience. 2006. Petition for Determination of Nonregulated Status for Glyphosate-Tolerant cotton: GlyTol™ cotton Event GHB614. Submitted by Alejandra Scott, Bayer CropScience USA LP, Research Triangle Park, North Carolina, 173pp. Retrieved on November 26, 2012 from http://www.aphis.usda.gov/biotechnology/not_reg.html.

Bayer CropScience. 2011. Liberty herbicide label, Cotton, pp. 12-14. Bayer CropScience LP, Research Triangle Park, North Carolina, 21 pp. Retrieved on November 2, 2012 from http://fs1.agrian.com/pdfs/Liberty_280_SL_Label2.pdf

Behrens MR, N Mutlu, S Chakraborty, R Dumitru, WZ Jiang, BJ LaVallee, PL Herman, TE Clemente and DP Weeks. 2007. Dicamba resistance: Enlarging and preserving biotechnology-based weed management strategies. *Science* 316:1185-1188.

Berg, G, N Roskot and K Smalla. 1999. Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. Journal of Clinical Microbiology 37:3594-3600.

Bevan, M, WM Barnes and M-D Chilton. 1983. Structure and transcription of the nopaline synthase gene region of T-DNA. Nucleic Acids Research 11:369-385.

Bi, JL, JB Murphy and GW Felton. 1997. Does salicylic acid act as a signal in cotton for induced resistance to *Helicoverpa zea*? Journal of Chemical Ecology 23(7):1805-1818.

Blasingame, D. 2010. Personal communication.

Bowman, DT, OA Gutierrez, RG Percy, DS Calhoun and OL May. 2006. Pedigrees of upland and pima cotton cultivars released between 1970 and 2005. Mississippi State University, Mississippi State, Mississippi. Available at <http://msucare.com/pubs/bulletins/b1155.pdf>, accessed November 5, 2012.

Brown JR. 2003. Ancient horizontal gene transfer. Nature Reviews Genetics 4:121-132.

Brubaker, CL, JA Koontz and JF Wendel. 1993. Bidirectional Cytoplasmic and Nuclear Introgression in the New World Cottons, *Gossypium barbadense* and *G. hirsutum* (Malvaceae). American Journal of Botany 80(10):1203-1208.

Buchanan, GA. 1992. Trends in weed control methods. Pages 47-72 in Weeds of Cotton: Characterization and Control. CG McWhorter and JR Abernathy (eds.). The Cotton Foundation, Memphis, Tennessee.

Chakraborty J, D Ghosal, A Dutta and TK Dutta. 2012. An insight into the origin and functional evolution of bacterial aromatic ring-hydroxylating oxygenases. Journal of Biomolecular Structure and Dynamics 30(1):1-19.

Chakraborty S, M Behrens, PL Herman, AF Arendsen, WR Hagen, DL Carlson, X-Z Wang and DP Weeks. 2005. A three-component dicamba *O*-demethylase from *Pseudomonas maltophilia*, strain DI-6: Purification and characterization. Archives of Biochemistry and Biophysics 437:20-28.

Chan, BG, AC Waiss Jr., RG Binder and CA Elliger. 1978. Inhibition of lepidopterous larval growth by cotton constituents. Entomologia Experimentalis et Applicata 24(3):294-300.

Clark, SE and GK Lamppa. 1992. Processing of the precursors for the light-harvesting chlorophyll binding proteins of photosystem II and photosystem I during import and in an organelle-free assay. Plant Physiology 98:595-601.

Coile, NC and MA Garland. 2003. Notes on Florida's Endangered and Threatened Plants. Florida Department of Agriculture and Consumer Services. Gainesville. Available at <http://www.virtualherbarium.org/EPAC/Notes2003.pdf>, accessed November 5, 2012.

Colson-Hanks, ES, SJ Allen and BJ Deverall. 2000. Effect of 2,6-dichloroisonicotinic acid or benzothiadiazole on *Alternaria* leaf spot, bacterial blight and *Verticillium* wilt in cotton under field conditions. *Australasian Plant Pathology* 29:170-177.

Crockett LJ. 1977. Wildly successful plants: A handbook of North American weeds. Honolulu, Hawaii: University of Hawaii Press, 609 pp.

D'Ordine, RL, TJ Rydel, MJ Storek, EJ Sturman, F Moshiri, RK Bartlett, GR Brown, RJ Eilers, C Dart, Y Qi, and others. 2009. Dicamba monooxygenase: Structural insights into a dynamic Rieske oxygenase that catalyzes an exocyclic monooxygenation. *Journal of Molecular Biology* 392:481-497.

Davies, AM, R Tata, RL Beavil, BJ Sutton, PR Brown. 2007. L-Methionine sulfoximine, but not phosphinothricin, is a substrate for an acetyltransferase (gene PA 4866) from *Pseudomonas aeruginosa*: structural and functional studies. *Biochemistry* 46:1829-1839.

DeJode, DR and JF Wendel. 1992. Genetic Diversity and Origin of the Hawaiian Islands Cotton, *Gossypium tomentosum*. *American Journal of Botany* 79(11):1311-1319.

Dixon RA. 2001. Natural products and plant disease resistance. *Nature* 411:843-847.

Dröge M, A Pühler and W Selbitschka. 1998. Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. *Journal of Biotechnology* 64:75-90.

Duke SO, J Lydon, WC Koshinen, TB Moorman, RL Chaney and R Hammerschmidt. 2012. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *Journal of Agricultural and Food Chemistry* 60:10375-10397.

Dumitru R, WZ Jiang, DP Weeks and MA Wilson. 2009. Crystal structure of dicamba monooxygenase: A Rieske nonheme oxygenase that catalyzes oxidative demethylation. *Journal of Molecular Biology* 392:498-510.

Echemendia, Y. 2010. Microorganism of the month: *Stenotrophomonas maltophilia*. Environmental Microbiology Laboratory, Inc., Cherry Hill, New Jersey. <http://www.emlab.com/s/sampling/env-report-07-2007.html>, accessed November 2, 2012.

Ellstrand NC, HC Prentice and JF Hancock. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30:539-563.

Fannin, B. 2010. Producers battling volunteer cotton in corn, sorghum fields. AgriLife Today, Texas A&M AgriLife, December 24, 2010. Available at <http://today.agrilife.org/2010/12/24/volunteer-cotton/>, accessed November 5, 2012.

Fraley, RT, SG Rogers, RB Horsch, PR Sanders, JS Flick, SP Adams, ML Bittner, LA Brand, CL Fink, JS Fry and others. 1983. Expression of bacterial genes in plant cells. Proceedings of the National Academy of Sciences of the United States of America 80:4803-4807.

Frear DS. 1976. The benzoic acid herbicides: IV - Dicamba and Tricamba. pp. 563-579. In PC Kearney and DD Kaufmann (eds), Herbicides: Chemistry, Degradation and Mode-of-Action – Second Edition. New York: Marcel Dekker.

Fryxell, PA. 1984. Taxonomy and Germplasm Resources. In: RJ Kohel and CF Lewis (Eds) Cotton, Agronomy Monograph 24, ASA-CSSA-SSA, Madison, WI, p27-57.

Goodfellow, M. and S.T. Williams. 1983. Ecology of actinomycetes. Annual Review of Microbiology 37:189-216.

Green, JM and MD Jones. 1953. Isolation of cotton for seed increase. Agronomy Journal 45(8):366-368.

Guinn, G. 1982. Causes of square and boll shedding in cotton. Technical Bulletin Number 1672. United States Department of Agriculture, Agricultural Research Services, Washington, D.C.

Hanson AD and S Roje. 2001. One-carbon metabolism in higher plants. Annual Review of Plant Physiology and Plant Molecular Biology 52: 119-137.

Heap I. 2012. International survey of herbicide resistant weeds. Retrieved on November 26, 2012 from <http://www.weedscience.org/in.asp>

Hegde SG, JD Nason, JM Clegg and NC Ellstrand. 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. Evolution 60(6):1187-1197.

Herman PL, M Behrens, S Chakraborty, BM Chrastil, J Barycki and DP Weeks. 2005. A three-component dicamba *O*-demethylase from *Pseudomonas maltophilia*, strain DI-6: Gene isolation, characterization, and heterologous expression. The Journal of Biological Chemistry 280(26):24759-24767.

H rouet, C, DJ Esdaile, BA Mallyon, E Debruyne, A Schulz, T Currier, K Hendrickx, R-J van der Klis and D Rouan. 2005. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regulatory Toxicology and Pharmacology 41:134-149.

- Herrmann KM. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. *Plant Cell* 7:907-919.
- Holm LG, JV Pancho, JP Herberger, DL Plucknett. 1979. *A Geographical Atlas of World Weeds*. John Wiley and Sons, New York, 391 pp.
- Hutmacher, RB, RN Vargas and SD Wright. 2006. *Methods to Enable the Coexistence of Diverse Cotton Production Systems*, University of California, Division of Agriculture and Natural Resources.
- ILSI-CERA. 2011. A review of the environmental safety of the PAT protein. International Life Sciences Institute, Center for Environmental Risk Assessment, Washington, D.C. Retrieved on November 2, 2012 from http://cera-gmc.org/docs/protein_monographs/pat_en.pdf.
- ILSI. 2010. International Life Sciences Institute, Crop Composition Database, Version 4.2. Retrieved on November 5, 2012 from www.cropcomposition.org
- Inbar, M, H Doostdar, D Gerling and RT Mayer. 2001. Induction of systemic acquired resistance in cotton by BTH has a negligible effect on phytophagous insects. *Entomologia Experimentalis et Applicata* 99:65-70.
- Jenkins, JN. 1993. Cotton. In *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology*. Paris, OECD (Organisation for Economic Co-Operation and Development): 61-70.
- Jiang, C-X, PW Chee, X Draye, PL Morrell, CW Smith and AH Paterson. 2000. Multilocus Interactions Restrict Gene Introgression in Interspecific Populations of Polyploid *Gossypium* (Cotton). *Evolution* 54(3):798-814.
- John, ME. 1996. Structural characterization of genes corresponding to cotton fiber mRNA, E6: Reduced E6 protein in transgenic plants by antisense gene. *Plant Molecular Biology* 30:297-306.
- Kalasz H. 2003. Biological role of formaldehyde, and cycles related to methylation, demethylation and formaldehyde production. *Mini Reviews in Medicinal Chemistry* 3(3): 175-192.
- Kämpfer, P. 2006. The family *Streptomycetaceae*, Part I: Taxonomy. Pages 538-604 in *The Prokaryotes. A Handbook on the Biology of Bacteria: Archaea. Bacteria: Firmicutes, Actinomycetes*. Volume 3. M Dworkin, S Falkow, E Rosenberg, K-H Schleifer, and E Stackebrandt (eds.). Springer+ Business Media, LLC., New York, New York.
- Kaneko T, Y Nakamura, S Sato, K Minamisawa, T Uchiumi, S Sasamoto, A Watanabe, K Idesawa, M Iriguchi, K Kawashima, M Kohara, M Matsumoto, S Shimpo, H Tsuruoka,

T Wada, M Yamada and S Tabata. 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Research 9: 189–197.

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

Keeler, K. 1989. Can Genetically Engineered Crops Become Weeds? Bio/Technology 7:1134-1139.

Keeling, JW., JD Reed, DM Petty and BA Brown. 2009. Control of volunteer Roundup Ready Flex cotton with postemergence herbicides. Page 1333 in 2009 Beltwide Cotton Conferences, San Antonio, Texas.

Keese P. 2008. Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research 7:123-149.

Kerby, TA, FM Bourland and KD Hake. 2010. Physiological rationales in plant monitoring and mapping. Pages 304-317 in Physiology of Cotton. JM Stewart, DM Oosterhuis, JJ Heitholt, and JR Mauney (eds.). Springer Science+Business Media, Dordrecht, Netherlands.

Klee HJ, YM Muskopf and CS Gasser. 1987. Cloning of an Arabidopsis thaliana gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: Sequence analysis and manipulation to obtain glyphosate-tolerant plants. Molecular and General Genetics 210:437-442.

Kong, G, MK Daud and S Zhu. 2010. Effects of pigment glands and gossypol on growth, development and insecticide-resistance of cotton bollworm (*Heliothis armigera* (Hubner)). Crop Protection 29:813-819.

Koonin EV, KS Makarova and L Aravind. 2001. Horizontal gene transfer in prokaryotes: Quantification and classification. Annual Review of Microbiology 55:709-742.

Krueger JP, RG Butz, YH Atallah and DJ Cork. 1989. Isolation and identification of microorganisms for the degradation of dicamba. Journal of Agricultural and Food Chemistry 37:534-538.

Kutzner, HJ. 1981. The family streptomycetaceae. Pages 2028-2090 in The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. Volume 2. MP Starr, H Stolp, HG Trüper, A Balows, and HG Schlegel (eds.). Springer-Verlag, Berlin, Germany.

Ledbetter, K. 2011. AgriLife extension specialist: Cotton planting should be delayed in times of drought. AgriLife Today, Texas A&M AgriLife, May 3, 2011. Available at

<http://today.agrilife.org/2011/05/03/agrilife-extension-specialist-cotton-planting-should-be-delayed-in-times-of-drought/>, accessed November 5, 2012.

Llewellyn, D, C Tyson, G Constable, B Duggan, S Beale, and P Steel. 2007. Containment of regulated genetically modified cotton in the field. *Agriculture, Ecosystems & Environment* 121(4):419-429.

Locci, R. 1989. Streptomycetes and related genera. Pages 2451-2508 in Bergey's Manual of Systematic Bacteriology. Volume 4. ST Williams and ME Sharpe (eds.). Williams & Wilkins, Baltimore, Maryland.

Maiti IB and RJ Shepherd. 1998. Isolation and expression analysis of peanut chlorotic streak caulimovirus (PCISV) full-length transcript (FLt) promoter in transgenic plants. *Biochemical and Biophysical Research Communications* 244:440-444.

Martinez, C, J-C Baccou, E Bresson, Y Baissac, J-F Daniel, A Jalloul, J-L Montillet, J-P Geiger, K Assigbetse and M Nicole. 2000. Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic responses of cotton challenged by an avirulent race of *Xanthomonas campestris* pv *malvacearum*. *Plant Physiology* 122:757-766.

McClelland, MR, KL Smith and JK Norsworthy. 2006. Managing glyphosate-resistant horseweed in conservation-tillage cotton production: Final summary and recommendations. AAES Research Series 552. University of Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

McGregor, SE. 1976. Insect Pollination of Cultivated Crop Plants. A. R. S. U.S. Department of Agriculture. Washington, DC, Government Printing Office.

Miki, B and S McHugh. 2004. Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *Journal of Biotechnology* 107:193-232.

Monsanto Company. 2010. Petition for the Determination of Nonregulated Status for Dicamba-Tolerant Soybean MON 87708. Submitted by R. Mannion, Monsanto Company, St. Louis, Missouri, 721 pp. Retrieved on November 26, 2012 from http://www.aphis.usda.gov/biotechnology/not_reg.html

Monsanto Company. 2004. Petition for the Determination of Nonregulated Status for MON 88017 Corn. Submitted by Ravinder Sinhu, Monsanto Company, St. Louis, Missouri, 278 pp. Retrieved on November 26, 2012 from http://www.aphis.usda.gov/biotechnology/not_reg.html.

Monsanto Company. 2012. Petition for the Determination of Nonregulated Status for Dicamba and Glufosinate-Tolerant Cotton MON 88701. Submitted by M. Malven, Monsanto Company, St. Louis, Missouri, 620 pp. Retrieved on May 28, 2012 from http://www.aphis.usda.gov/biotechnology/not_reg.html

Morgan, GD, DA Fromme, PA Baumann, J Grichar, B Bean, ME Matocha and DA Mott. 2011. Managing volunteer cotton in grain crops (corn, sorghum, soybean, and wheat). Texas AgriLife Extension Service, Texas A&M University. Available at http://publications.tamu.edu/COTTON/PUB_cotton_Managing%20Volunteer%20Cotton%20in%20Grain%20Crops.pdf, accessed November 5, 2012.

Muenscher, WC. 1980. Weeds. Cornell University Press, New York and London.

Münster, P and AM Wieczorek. 2007. Potential gene flow from agricultural crops to native plant relatives in the Hawaiian Islands. *Agriculture, Ecosystems & Environment* 119(1-2):1-10.

National Cotton Council. 2011. National Cotton Council Disease Database. Retrieved November 26, 2012 from <http://www.cotton.org/tech/pest/>.

Niepel M and DR Gallie. 1999. Identification and characterization of the functional elements within the tobacco etch virus 5' leader required for cap-independent translation. *Journal of Virology* 73:9080-9088.

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

OECD. 1999. Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. ENV/JM/MONO(99)13. Organisation for Economic Co-operation and Development, Paris, France, 26 pp. At <http://www.oecd.org/science/biosafety-biotrack/46815618.pdf>, accessed November 2, 2012.

OECD. 2002. Module II: Herbicide biochemistry, herbicide metabolism and the residues in glufosinateammonium (Phosphinothricin)-tolerant transgenic plants. ENV/JM/MONO(2002)14. Organisation for Economic Co-operation and Development, Paris, France, 22 pp. At <http://www.oecd.org/science/biosafety-biotrack/46815748.pdf>, accessed November 2, 2012.

OECD. 2008. Consensus document on the biology of cotton (*Gossypium* spp.). ENV/JM/MONO(2008)33. Organisation for Economic Co-operation and Development, Paris, France, 64 pp. At <http://www.oecd.org/science/biosafety-biotrack/46815918.pdf>, accessed November 2, 2012.

OECD. 2009. Consensus document on the compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2004)16. Organisation for Economic Co-operation and Development, Paris, France, 32 pp. At <http://www.oecd.org/science/biosafety-biotrack/46815236.pdf>, accessed November 2, 2012.

OGTR. 2008. The Biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton), version 2. Department of Health and Ageing, Office of the Gene Technology Regulator, Government of Australia. Available at [http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/cotton-3/\\$FILE/biologycotton08.pdf](http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/cotton-3/$FILE/biologycotton08.pdf), accessed November 5, 2012.

Palleroni NJ and JF Bradbury. 1993. *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983. International Journal of Systematic Bacteriology 43(3):606-609.

Park S-W, P-P Liu, F Forouhar, AC Vlot, L Tong, L Tietjen and DF Klessig. 2009. Use of a salicylic acid analog to investigate the roles of methyl salicylate and its esterases in plant disease resistance. The Journal of Biological Chemistry 284(11):7307-7317.

Percival, AE, JF Wendel and JM Stewart. 1999. Taxonomy and germplasm resources. Pages 33-63 in Cotton: Origin, History, Technology, and Production. W.C. Smith and J.T. Cothren (eds.). John Wiley & Sons, Inc., New York, New York.

Percy, RG and JF Wendel. 1990. Allozyme evidence for the origin and diversification of *Gossypium barbadense* L. Theoretical and Applied Genetics 79:529-542.

Peterson, C.D., Pearman, D. A., and Dines, T. D. 2002. New Atlas of the British flora. Oxford University Press, London, U.K.

Pleasants, JM and JF Wendel. 2005. Assessment of potential for gene flow between transgenic cotton and the endemic Hawaiian cotton - Final Report. EPA Grant R-83032901.

Pleasants, JM and JF Wendel. 2010. Reproductive and Pollination Biology of the Endemic Hawaiian Cotton, *Gossypium tomentosum* (Malvaceae). Pacific Science 64(1):45-55.

Rani, PR and P Rajasekharreddy. 2010. Insecticidal activity of (2*n*-octylcycloprop-1-enyl)-octanoic acid (I) against three coleopteran stored product insects from *Sterculia foetida* (L.). Journal of Pest Science 83:273-279.

Rensing SA and U-G Maier. 1994. Phylogenetic analysis of the stress-70 protein family. Journal of Molecular Evolution 39:80-86.

Rieseberg LH. 1997. Hybrid origins of plant species. Annual Review of Ecology and Systematics 28:359-389.

Rieseberg LH and JF Wendel. 1993. Introgression and its consequences in plants. pp. 70-109. In RG Harrison (ed), Hybrid Zones and the Evolutionary Process. Oxford, U.K.: Oxford University Press.

Roberts, G, S Kerlin and M Hickman. 2002. Controlling volunteer cotton. Page F4 in WEEDpak – A Guide for Integrated Management of Weeds in Cotton. Cotton Catchment Communities CRC, Australian Cotton Research Institute, Narrabri, New South Wales. Retrieved from http://www.cottoncrc.org.au/industry/Publications/Weeds/WEEDpak/F1_Farm_Hygiene, November 5, 2012.

Rodoni S, W Mühlecker, M Anderl, B Kräutler, D Moser, H Thomas, P Matile and S Hörtensteiner. 1997. Chlorophyll breakdown in senescent chloroplasts: Cleavage of pheophorbide a in two enzymic steps. *Plant Physiology* 115:669-676.

Saha, S, DA Raska and DM Stelly. 2006. Upland Cotton (*Gossypium hirsutum* L.) x Hawaiian Cotton (*G. tomentosum* Nutt. ex Seem.) F₁ Hybrid Hypoaneuploid Chromosome Substitution Series. *Journal of Cotton Science* 10:263-272.

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

Sardi E, J Velich and J Szarka. 1996. The presence of Selye's stress-syndrome in the Snap Bean – Halo Blight relationship. Reports of Bean Improvement Cooperative and National Dry Bean Council Research Conference, Annual Report 39:278-279.

Silverman FP, PD Petracek, DF Heiman, CM Fledderman and P Warrior. 2005. Salicylate activity. 3. Structure relationship to systemic acquired resistance. *Journal of Agricultural and Food Chemistry* 53(25):9775-9780.

Soltis DE and PS Soltis. 1993. Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* 12:243-273.

Stace, C.A. 1987. Hybridization and the plant species. Pp. 115–127 in K.M. Urbanska (ed.) *Differentiation Patterns in Higher Plants*. Academic Press, New York.

Szende B and E Tyihak. 2010. Effect of formaldehyde on cell proliferation and death. *Cell Biology International* 34:1273-1282.

Thaler, JS, AA Agrawal and R Halitschke. 2010. Salicylate-mediated interactions between pathogens and herbivores. *Ecology* 91(4):1075-1082.

Thaler, JS, PT Humphrey and NK Whiteman. 2012. Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Sciences* 17(5):260-270.

Thompson, A and L Steckel. 2010. Controlling volunteer cotton in soybeans. W168. University of Tennessee Extension, Memphis, Tennessee. Retrieved from <https://utextension.tennessee.edu/publications/Documents/W168.pdf>, accessed November 5, 2012.

Thompson, CJ, NR Movva, R Tizard, R Cramer, JE Davies, M Lauwereys and J Botterman. 1987. Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. EMBO Journal 6:2519-2523.

Umbeck, PF, KA Barton, EV Nordheim, JC McCarty, WL Parrott and JN Jenkins. 1991. Degree of pollen dispersal by insects from a field test of genetically engineered cotton. Journal of Economic Entomology 84:1943-1950.

University of California. 1996. Development and growth requirements of the cotton plant. Pages 11-23 in Integrated Pest Management for Cotton in the Western Region of the United States. Publication 3305. Second Edition. University of California, Oakland, California.

USDA-APHIS. 2012. United States Department of Agriculture, Animal Plant Health Inspection Service. Federal Noxious Weed List as of 2/1/2012. Retrieved November 5, 2012 from http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf

USDA-ERS-FAS. 2010. Outlook for U.S. agricultural trade: FY 2010 exports expected to rise to \$104.5 billion; Imports drop to \$76.5 billion. U.S. Department of Agriculture, Economic Research Service and Foreign Agricultural Service, Washington, D.C. http://www.fas.usda.gov/cmp/outlook/2010/May-10/May2010_fullreport.pdf, accessed November 5, 2012.

USDA-NASS. 2012a. United States Department of Agriculture, National Agricultural Statistics Service. Crop Production, pp. 14-15. Retrieved on November 5, 2012 from <http://usda01.library.cornell.edu/usda/nass/CropProd//2010s/2012/CropProd-05-10-2012.pdf>.

USDA-NASS. 2012b. United States Department of Agriculture, National Agricultural Statistics Service. National Statistics for Cotton. Cotton County Maps: Planted Acreage By County. Retrieved on November 26, 2012 from http://www.nass.usda.gov/Statistics_by_Subject/result.php?6AC9DE2A-0B22-34E4-BC77-4797B1AA5DAD§or=CROPS&group=FIELD%20CROPS&comm=COTTON

USDA-NRCS. 2012a. Plant profile for *Gossypium hirsutum* L. The PLANTS Database. National Plant Data Team, Greensboro, NC. At <http://plants.usda.gov>, accessed November 26, 2012.

USDA-NRCS. 2012b. United States Department of Agriculture, Natural Resources Conservation Service. Invasive and Noxious Weeds. At <http://plants.usda.gov/java/invasiveOne?startChar=G>, accessed November 26, 2012.

USDA-NRCS. 2012c. United States Department of Agriculture, Natural Resources Conservation Service. Native, Invasive, and Other Plant-Related Definitions. At http://www.ct.nrcs.usda.gov/plant_definitions.html, accessed November 19, 2012.

USDA-NRCS. 2012d. Plant profile for *Gossypium barbadense*. The PLANTS Database. National Plant Data Team, Greensboro, NC. At <http://plants.usda.gov>, accessed November 26, 2012.

USDA-NRCS. 2012d. Plant profile for *Gossypium thurberi*. The PLANTS Database. National Plant Data Team, Greensboro, NC. At <http://plants.usda.gov>, accessed November 26, 2012.

USDA-NRCS. 2012f. Plant profile for *Gossypium tomentosum*. The PLANTS Database. National Plant Data Team, Greensboro, NC. At <http://plants.usda.gov>, accessed November 26, 2012.

U.S. EPA. 1997. Phosphinothricin acetyltransferase and the genetic material necessary for its production in all plants; Exemption from the requirement of a tolerance on all raw agricultural commodities. Federal Register 62:17717-17720.

U.S. EPA. 2003. Memorandum: Glufosinate ammonium (PC code 128850). Section 3 registrations for transgenic cotton and cotton (ID# - 0F06140), transgenic rice (ID# - 0F06210), and bushberry (ID# - 2E06404). Human health risk assessment. DP barcode: D290086. Case number: 293386. Submission: S635308. 40 CFR 180.473. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C.

U.S. EPA. 2008. Glufosinate final work plan registration review: August 2008. Docket Number: EPA-HQ-OPP-2008-0190. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2009. Environmental Protection Agency. Reregistration Eligibility Decision for Dicamba and Associated Salts (Amended). EPA, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. Retrieved on May 29, 2012 from <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2005-0479-0026>

U.S. FDA. 1998. Guidance for industry: use of antibiotic resistance marker genes in transgenic plants. Draft guidance. US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Center for Veterinary Medicine. September 4, 1998. Available at <http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096135.htm>, accessed November 5, 2012.

U.S. FDA. 2011. Food and Drug Administration, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition. Completed Consultations on Bioengineered Foods: BNF No. 125. Retrieved on May 29, 2012 from

<http://www.fda.gov/Food/Biotechnology/Submissions/ucm282993.htm> and
<http://www.fda.gov/Food/Biotechnology/Submissions/ucm282994.htm>

Van Deynze, AE, RB Hutmacher and KJ Bradford. 2011. Gene flow between *Gossypium hirsutum* L. and *Gossypium barbadense* L. is asymmetric. *Crop Science* 51:298-305.

Van Deynze, AE, FJ Sundstrom, and KJ Bradford. 2005. Pollen-Mediated Gene Flow in California Cotton Depends on Pollinator Activity. *Crop Science* 45(4):1565-1570.

Vencill W, RL Nichols, TM Webster, J Soteres, C Mallory-Smith, NR Burgos, WG Johnson and MR McClelland. 2012. Herbicide resistance: Toward an understanding of resistance development and the impact of herbicide-resistant crops. *Weed Science* 60 (Special Issue). Available at <http://www.wssajournals.org/doi/pdf/10.1614/WS-D-11-00206.1>, accessed May 29, 2012.

Vlot AC, DA Dempsey and DF Klessig. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology* 47:177:206.

Wang X-Z, B Li, PL Herman and DP Weeks. 1997. A three-component enzyme system catalyzes the *O* demethylation of the herbicide dicamba in *Pseudomonas maltophilia* DI-6. *Applied and Environmental Microbiology* 63(4):1623-1626.

Webster, TM and RL Nichols. 2012. Changes in the prevalence of weed species in the major agronomic crops of the southern United States: 1994/1995 to 2008/2009. *Weed Science*. 60(2):145-157. Available at <http://www.bioone.org/doi/full/10.1614/WS-D-11-00092.1>, accessed November 2, 2012.

Wehrmann, A, AV Vliet, C Opsomer, J Botterman and A Schulz. 1996. The similarities of *bar* and *pat* gene products make them equally applicable for plant engineers. *Nature Biotechnology* 14:1274-1278.

Wendel, JF, CL Brubaker and AE Percival. 1992. Genetic Diversity in *Gossypium hirsutum* and the Origin of Upland Cotton. *American Journal of Botany* 79(11):1291-1310.

WHO-IPCS. 1989. World Health Organization, International Programme on Chemical Safety. Environmental health criteria 89: Formaldehyde. Retrieved on June 24, 2012 from <http://www.inchem.org/documents/ehc/ehc/ehc89.htm>

Williams, MR. 2007. Cotton Losses – 2007. Table 7 – Summary of all states. Mississippi State University Extension Service, Mississippi State University. Retrieved on November 20, 2012 from <http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/>.

Williams, MR. 2008. Cotton Losses – 2008. Table 6 – Summary of all states. Mississippi State University Extension Service, Mississippi State University. Retrieved on November 20, 2012 from <http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/>.

Williams, MR. 2009. Cotton Losses – 2009. Table 8 – Summary of all states. Mississippi State University Extension Service, Mississippi State University. Retrieved on November 20, 2012 from <http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/>.

Williams, MR. 2010. Cotton Losses – 2010. Table 8 – Summary of all states. Mississippi State University Extension Service, Mississippi State University. Retrieved on November 20, 2012 from <http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/>.

Williams, MR. 2011. Cotton Losses – 2011. Table 8 – Summary of all states. Mississippi State University Extension Service, Mississippi State University. Retrieved on November 20, 2012 from <http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/>.

Winter, J, R Wright, N Duck, C Gasser, R Fraley and D Shah. 1988. The inhibition of petunia hsp70 mRNA processing during CdCl₂ stress. *Molecular and General Genetics* 211:315-319.

Wood DW, JC Setubal, R Kaul, DE Monks, JP Kitajima et al. 2001. The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science* 294:2317-2323.

WSSA. 1998. Weed Science Society of America. Resistance and tolerance definitions. *Weed Technology* 12(4):789. Retrieved on August 10, 2012 from <http://www.wssa.net/Weeds/Resistance/definitions.htm>

Wunderlin, RP and BF Hansen. 2008. Atlas of Florida Vascular Plants, [SM Landry and KN Campbell (application development), Florida Center for Community Design and Research.] Institute for Systematic Botany, University of South Florida, Tampa.

Yang M, E Wardzala, GS Johal and J Gray. 2004. The wound-inducible *Lls1* gene from maize is an orthologue of the *Arabidopsis Acd1* gene, and the LLS1 protein is present in non-photosynthetic tissues. *Plant Molecular Biology* 54: 175-191.

York, AC, AM Stewart, PR Vidrine and AS Culpepper. 2004. Control of volunteer glyphosate resistant cotton in glyphosate-resistant soybean. *Weed Technology* 18:532-539.

Yoshida S, S Maruyama, H Nozaki and K Shirasu. 2010. Horizontal gene transfer by the parasitic plant *Striga hermonthica*. *Science* 328:1128.

Zarate, SI, LA Kempema and LL Walling. 2007. Silverleaf whitefly induces salicylic acid defense and suppresses effectual jasmonic acid defenses. *Plant Physiology* 143:866-875.

Zhang, B-H, X-P Pan, T-L Guo, Q-L Wang and TA Anderson. 2005. Measuring gene flow in the cultivation of transgenic cotton (*Gossypium hirsutum* L.). *Molecular Biotechnology* 31:11-20.

Zhang, P, X Zhu, F Huang, Y Liu, J Zhang, Y Lu and Y Ruan. 2011. Suppression of jasmonic acid-dependent defense in cotton plant by the mealybug *Phenacoccus solenopsis*. PLoS ONE 6(7): e22378. doi:10.1371/journal.pone.0022378.