Texas A&M Petition (17-292-01p) for Determination of Non-regulated Status of Ultra-Low Gossypol Cottonseed TAM66274

OECD Unique Identifier: TAM-66274-5

Draft Plant Pest Risk Assessment

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A. Introduction

Texas A&M AgriLife Research, Texas A&M University 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) cotton (*Gossypium hirsutum* L. Merr.) event TAM66274 under OECD Unique Identifier TAM-66274-5 (hereafter referred to as TAM66274 cotton) is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived 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 17-292-01p, and is hereafter referenced as Texas A&M 2017. 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. 7702 *et seq*.)[1]. This plant pest risk assessment was conducted to determine if TAM66274 cotton is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

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 part 340 when APHIS determines that it is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. A GE organism is considered a regulated article under part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[2]. TAM66274 cotton was produced by the *Agrobacterium tumefaciens*-mediated transformation of cotyledon, cotyledonary-petiole and hypocotyl segments of 10-day-old cotton (*G. hirsutum* cv. Coker 312) seedlings (p. 39, Texas A&M 2017), and five inserted construct components come from a plant pest organism listed in 7 CFR 340.2, e.g. *A. tumefaciens*, including seven nucleotides of the 25 base pairs of the left border T-DNA repeat, 51 nucleotides of the 5' end of the nopaline synthase (*nos*) gene, *nos* gene promoter and terminator, and octopine synthase (*ocs)* gene terminator (Table 4-5 and Figure 4-14, pp.72-74, Texas A&M 2017). Therefore, the GE cotton Event TAM66274 is considered a regulated article under APHIS regulations at 7 CFR part 340. Texas A&M has conducted introductions of TAM66274 cotton as a regulated article under APHIS-authorized notifications since 2009 (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/sa_permits/ct_status), in part, to gather information to support that TAM66274

[1] 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."

[2] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2.(b).

cotton is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with TAM66274 cotton and its progeny and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if TAM66274 cotton is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about TAM66274 cotton related to plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on nontarget organisms; 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; 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 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with 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.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq)* 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. 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) (21 U.S.C. §301 et seq.). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs) and part 172 - Experimental Use Permits. No EPA reviews are relevant to TAM66274 cotton.

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 GE crops comply with

their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (FDA 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (FDA, 1992). Texas A&M initiated food safety consultations with FDA in 2012 in accordance with FDA's policy statement and industry guidance. Texas A&M has prepared a safety and nutritional assessment of food and feed derived from TAM66274 cotton and expects to submit its findings to FDA soon.

B. Development of TAM66274 Cotton

Cotton belongs to the genus *Gossypium*, which consists of 50 species, of which four are domesticated species, including the two New World allotetraploids *G. hirsutum* and *G. barbadense* (2n = 4x = 52), and the two Old World diploids *G. arboretum* and *G. herbaceum* (2n = 2x = 26) (OECD 2008; Wendel et al. 2010). *G. hirsutum*, known as upland cotton, is the most widely grown species worldwide, which is grown on over 95% of the world-wide cotton hectarages (OECD 2008; Freeland et al. 2010). In the United States, only the two New World domesticated allotetraploid cotton species, upland cotton (*G. hirsutum*) and *G. barbadense* (known as Pima or Egyptian cotton), are cultivated commercially and in 2017, they comprised 98% and 2% of all planted acres of cotton, respectively (USDA-NASS 2017). Upland cotton (*G. hirsutum*) is the subject of this risk assessment not only because it is the predominant type grown in U.S. but also because TAM66274 cotton was derived from *G. hirsutum* cv. Coker 312.

Cultivated cotton is grown as an annual crop although it can be a perennial in tropical areas. Cotton is sensitive to low temperatures, and its growth cycle generally needs more than 150 days above 15°C. The temperatures below 15°C will cause growth arrest and the freezing temperature even kills cotton plants (OECD 2008; Freeland et al. 2010). Thus, temperature is the main climatic factor restricting the geographic distribution of cotton crop. Cotton is primarily grown between 37º N and 32º S although it is also grown up to 45º N (OECD 2008; Freeland et al. 2010).

In the United States, cotton is grown in 17 states in Southern and Southwestern regions, with major concentrations in the Texas High and Rolling Plains; the Mississippi, Arkansas and Louisiana Delta; Southern Georgia; and California's San Joaquin Valley (Figure 1, colored areas) (USDA-NASS 2017a). As shown in Figure 2, there exists a significant year-to-year variability in planted acreages, ranging from 8-16 million acres in the past 20 years (USDA-NASS 2017b). Cotton yields (pounds/acre) also differ from year to year but show an apparent increase (about 40%) over the years (Figure 3) (USDA-NASS 2017c).

Cotton is primarily used for its textile fibers, and it is the world's leading source for natural fibers (OECD 2008). Other than the fiber, cotton seed is a rich source of oil and protein, which are about 21% and 23% of the seed weight, respectively (Liu et al. 2012). Thus, cotton seeds add high values to cotton crop by providing cottonseed oil for human

consumption and cottonseed meal for livestock (OECD 2008, 2009). Cottonseed oil production ranks fifth among the oil seed crops in the past five years, behind soybean, rapeseed, sunflower, and peanut (USDA-FAS 2017). The cotton seed-derived protein meal ranks fourth behind the protein-meals derived from soybean, rapeseed, and sunflower (USDA-FAS 2017). Therefore, cottonseed is an economically important secondary product of cotton production, and it accounts for between 12-24% of crop value (Liu et al. 2012; USDA-ERS 2017).

Figure 1. Cotton production areas in the U.S. (USDA NASS 2016). All the 17 cotton production states are highlighted in color. For each cotton production state, the number on top represents the number of acres planted to cotton in 2017 while the number at the bottom represents the increased or decreased (minus) acres of planted cotton states in 2017 compared to that in 2016.

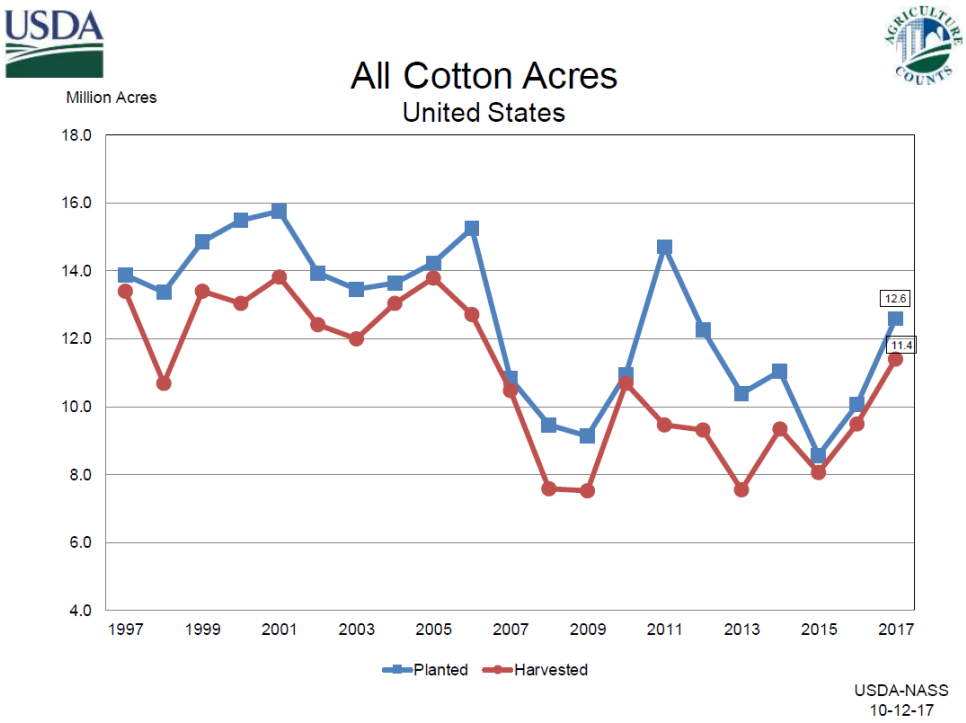

Figure 2. Cotton acreage by year in the U.S. (USDA NASS 2017)

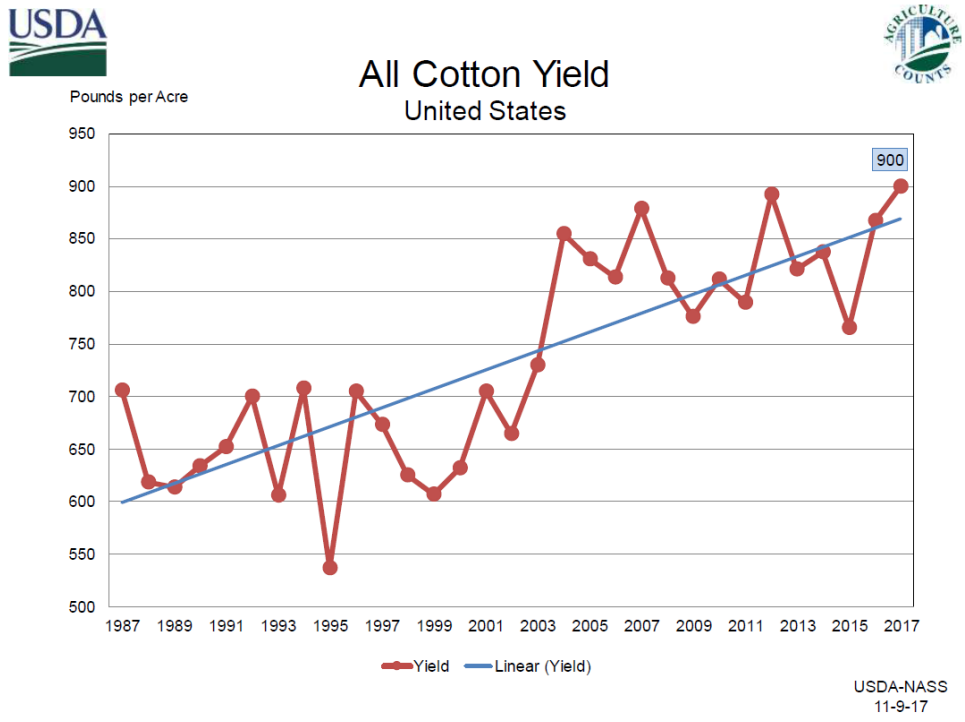

Figure 3. Cotton yield by year in the U.S. (USDA NASS 2017)

Despite its high content of protein and usage as a feed supplement for ruminant animals, cotton seed is not normally consumed by humans or monogastric animals due to the presence of toxic gossypol (Gadelha et al. 2014). For the safe use of cottonseed products in human food, FDA set the safety standard as less than 450 ppm of free gossypol content (FDA 1960, 1972). Similarly, for the safe use of cottonseed products as monogastric animal feed, the Association of American Feed Control Officials (AAFCO) established the safety standard as less than 400 ppm of free gossypol content (AAFCO 1968). The gossypol content in in cottonseed of parental variety cv. Coker 312 is over 10,000 ppm (Texas A&M 2017), which is much higher than the above established safety standard levels, and thus limits the use of cottonseed for human consumption and animal feed.

Long-lasting efforts have been made to reduce gossypol level in cottonseed (Sunilkumar et al. 2006; Liu et al. 2012). While a variety of processing approaches such as air classification, liquid cyclone processing, solvent extraction, and screwpress techniques prove to be successful to reduce the free gossypol level below the safety standards level, they all are cost prohibitive. The breeding method to reduce cottonseed gossypol level was developed after the discovery of a glandless mutant, which lacked glands where gossypol are stored (McMichael 1959). The glandless trait has been transferred through breeding into new cotton varieties to produce gossypol-free cottonseed. However, gossypol and related terpenoids present in glands are present on the surface of the vegetative and floral parts of the plant and they play an important protective role against pests and some diseases. Therefore, despite their beneficial food value these glandless cotton varieties are very susceptible to a host of insect pests and diseases under field conditions, making them economically less viable (Sunilkumar et al. 2006).

TAM66274 cotton was developed by *A. tumefaciens*-mediated transformation of cotton seedling tissues from non-transgenic upland cotton cv. Coker 312 using plasmid pART27-LCT66 (Texas A&M 2017). The pART27-LCT66 vector contains an RNA interference (RNAi) expression cassette, which is designed to suppress the expression of δ-cadinene synthase (*dCS*) gene that encodes δ-cadinene synthase (dCS), a key enzyme involved in gossypol biosynthesis. The RNAi-mediated suppression of *dCS* gene expression with a seed specific promoter leads the TAM66274 cotton to produce ultra-low levels of gossypol only in the seed, while maintaining normal plant-protecting gossypol levels in the rest of the plant. Such an approach presents a very promising way to reduce gossypol only in cottonseed so that this valuable source of protein may be used in human food and animal feed applications. Texas A&M expects the ultra-low gossypol trait in TAM66274 cotton will be used in commercial varieties as a stand-alone trait in the short term but will be eventually stacked with other important traits in new cotton varieties.

The genetic engineering and breeding steps for the development of TAM66274 cotton are described in the petition (Figures 3-4 and 5, pp. 40-41; Texas A&M 2017). Southern blot analysis showed that a single intact copy of T-DNA was integrated in TAM66274 cotton, which consists of the *dCS* gene RNAi cassette and the *nptII* gene variant cassette. The structural stability of the T-DNA insert in TAM66274 cotton was confirmed by Southern blot analysis on individual plants across three different generations (T1, T2, and T3).

PCR and sequencing analyses of the genomic DNA regions flanking the inserted T-DNA demonstrated that the T-DNA integration occurred in the last intron of a putative *α-hydrolase* gene (Texas A&M 2017).

Compositional analyses of TAM662741 cotton were compared to its parental cotton variety, Coker 312, in replicated field trials at three and five locations in the U.S. during the summers of 2014 and 2015, respectively. All the field sites represent major cotton growing regions in the United States (Texas A&M 2017). The compositional analyses were also compared to conventional nutrient and anti-nutrient ranges found in the International Life Sciences Institute database (ILSI 2016). The purpose of the compositional analyses of TAM66274 cotton was two-fold: to evaluate if the cotton seed gossypol levels in TAM66274 cotton meet the safety standards established for intended food and feed uses; and to compare the nutrient and anti-nutrient levels in cottonseed of TAM66274 cotton to that of both control variety cv. Coker 312 and published values for other conventional cotton varieties.

Texas A&M also evaluated 40 phenotypic, agronomic and ecological characteristics of TAM66274 by comparing with its parental control variety, cv. Coker 312 under field conditions at eight locations representing major U.S. cotton growing regions during 2014 and 2015 cotton growing seasons (Table 7-3, pp. 148-149, Texas A&M 2017).

Based on cotton biology (Fryxell 1979; OECD 2008; OGTR 2008; Wendel et al. 2010; Lee and Fang 2015) and the data presented by the petitioner (Texas A&M 2017), APHIS concludes that TAM66274 cotton was developed in a manner common to other GE cotton and GE crops using *Agrobacterium*-mediated transformation (USDA-APHIS-BRS 2017).

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNAs and their expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in TAM66274 cotton relative to the parental control variety and other cotton comparator varieties. The assessment encompasses a consideration of the *dCS* RNAi cassette that silences *dCS* genes encoding dCS enzyme involved in gossypol synthesis, neomycin phosphotransferase II (NPTII) variant protein encoded by *nptII* gene variant, and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested seeds

derived from the GE crop event compared to those in the conventional counterpart and other comparators.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the GE crop event; or for expression of inserted DNAs, new proteins or enzymes, or changes in metabolism to affect plant pests or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pests or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

TAM66274 cotton was developed through *Agrobacterium*-mediated transformation of cotton variety cv. Coker 312 using the disarmed pART27-LCT66 binary vector (Section 3, Texas A&M 2017). The disarmed binary vector does not have the native transferred DNA (T-DNA) region from tumor-inducing (Ti) plasmids normally responsible for the incitation of crown gall tumors upon *A. tumefaciens* infection (Gelvin 2003). Furthermore, following transformation the explant tissues were placed on selection medium supplemented with antibiotic carbenicillin to arrest the growth of *Agrobacterium* (p.39, Texas A&M 2017).

Binary Plasmid Vector pART27-LCT66

The disarmed pART27-LCT66 binary vector is approximately 15.6 kb. It contains two gene expression cassettes which are delineated by a right border (RB) and left border (LB) sequences of T-DNA as well as backbone vector sequences outside of the two T-DNA border sequences. Figure 3-2 and Table 3-1 (pp. 34-39, Texas A&M 2017) lists all genetic elements in the binary plasmid vector pART27-LCT66. Transgene elements within the T-DNA regions are shown in Figure 4 below.

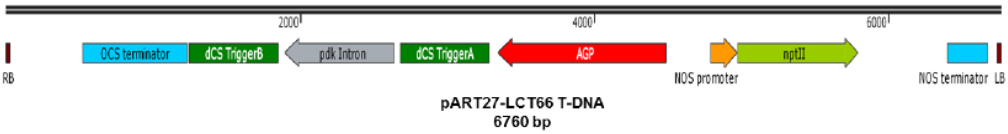

Figure 4. Transgene elements within the T-DNA regions (Figure 3-3, p.34, Texas A&M 2017).

- The first gene cassette, i.e. *dCS* RNAi, is comprised of a highly seed-specific α-globulin B gene promoter (AGP) from cotton (*G. hirsutum*), a 604 bp internal sequence (Trigger A) of the *dCS* gene from cotton (*G. hirsutum*), an intron of the pyruvate orthophosphate dikinase (*pdk*) gene from *Flaveria trinervia*, a reverse complement of the Trigger A sequence (Trigger B), and the octopine synthase (*ocs)* gene terminator from *A. tumefaciens*. The trigger A sequence has over 80% homology to other

sequences of cotton *dCS* genes. The expression of dCS gene cassette is intended to silence multiple members of the *dCS* gene family in cotton.

- The second expression cassette contains the nopaline synthase (*nos*) gene promoter and terminator from *A. tumefaciens*, selectable marker gene *nptII* variant from *E. coli*, and 51 nucleotides of the 5' end of the *nos* gene fused with *nptII* gene. This gene cassette confers antibiotic (kanamycin) resistance, allowing selection of transformed cells in tissue culture.

Among the above transgene elements within the T-DNA regions, six elements are derived from *A. tumefaciens*, which is listed as a plant pathogen. These six elements include the T-DNA left and right border sequences, 51 nucleotides of the 5' end of the nopaline synthase (*nos*) gene fused with *nptII* gene, *ocs* gene terminator, *nos* gene promoter and terminator. However, none of them is known to cause plant diseases.

Characteristics, Stability, and Inheritance of the Introduced DNA

Texas A&M has provided data to characterize the inserted transgene DNAs in TAM66274 cotton with a combination of techniques, including Southern blot analysis, PCR, and DNA sequencing (Section 4, pp.43-81, Texas A&M 2017). Southern blot analyses demonstrate that TAM66274 cotton genome does not contain any binary vector backbone elements outside of the T-DNA regions (pp.44-50, Texas A&M 2017). Furthermore, Southern blot analyses using probes corresponding to the RB, LB, and internal genetic elements of the T-DNA confirmed that a single intact copy of the T-DNA was integrated in TAM66274 cotton (pp. 51-62, Texas A&M 2017). Genomic DNA sequences flanking the inserted T-DNA were determined using HE-TAIL PCR followed by DNA sequencing of the PCR products. The flanking sequence analyses showed that the entire 25 bp RB T-DNA repeat (plus three bp of the RB overdrive) and 18 bp of the 25 bp LB T-DNA repeat were absent in TAM66274 cotton (pp. 62-69, Texas A&M 2017). The organization and integrity of the genetic elements within the inserted T-DNA were confirmed using overlap PCR followed by DNA sequence analysis of the amplified products, and the data showed that the organization and sequence of each of the genetic elements in the T-DNA in TAM66274 are identical to those in plasmid pART27- LCT66, confirming that a single copy of T-DNA was inserted in TAM66274 cotton (pp. 70-74, Texas A&M 2017).

The bioinformatics analyses of the T-DNA genomic flanking sequences in TAM66274 cotton revealed that the T-DNA was inserted within the last intron of an α-*hydrolase* gene, which is located on Chromosome D7. It was also shown that a 44 bp of cotton nucleotide sequence was deleted during the T-DNA insertion. However, according to the petitioner, this deletion would not result in disruption to any known or putative genes (pp.75-77, Texas A&M 2017).

The stability of the transgene integration in TAM66274 cotton genome was demonstrated over three breeding generations (T1, T2 and T3) using Southern blot analyses. Furthermore, Texas A&M also assessed T-DNA inheritance in three segregating populations, including the T2 generation of TAM66274 cotton, a BC_1F_2 generation and a

F_2 generation of the cross Stoneville 474/TAM66274 cotton, and showed that the T-DNA is inherited as a single locus according to Mendel's principles of inheritance. These results further confirmed that the T-DNA is stably integrated in TAM66274 cotton at a single chromosomal locus (pp.781-80, Texas A&M 2017).

Expression of inserted DNA, changes in gene expression, new proteins or metabolism, and toxicity and allergenicity

As described above, the inserted T-DNA in TAM66274 cotton contains two gene cassettes, i.e. *dCS* RNAi and *nptII* gene variant.

dCS RNAi cassette

All cotton species have characteristic pigment glands throughout the cotton plant, such as foliage, floral organs, bolls, roots and seeds, which contain terpenoids including the sesquiterpenoid gossypol (Cai et al. 2010). A pathway for the biosynthesis of terpenoids including gossypol in cotton has been proposed, as illustrated in Figure 5. The cotton sesquiterpenes (C15) are derived via the mevalonate pathway (Heinstein et al. 1970). One early and key step of cotton terpenoid including gossypol biosynthesis is to cyclize farnesyl diphosphate (FDP), the linear carbon skeleton of the sesquiterpenes in cotton, to (+)-δ-cadinene through δ-cadinene synthase (dCS) enzyme.

The *dCS* RNAi cassette in TAM66274 cotton was designed to specifically silence the endogenous *dCS* genes in cottonseed by using the seed-specific α-globulin B gene promoter (AGP) while leaving the expression of *dCS* genes unchanged in other parts of the cotton plant. The expression of this cassette in TAM66274 cotton leads to the formation of a hairpin double-stranded RNA (dsRNA) structure with a stem formed between the complementary cotton *dCS* gene sequences (Trigger A and B) and a loop from the *pdk* gene intron. This hairpin/stem-loop structure will be recognized and processed by the cotton plant's RNAi machinery, resulting in suppression of expression of the *dCS* genes in cottonseed. The trigger sequence has 80.9–99.8% homology to several other published sequences of *dCS* genes from the diploid (*G. arboreum*) and tetraploid (*G. hirsutum*) cottons (Sunilkumar et al. 2006). Thus, this sequence was intended to target all members of the *dCS* gene family because it bears several stretches (20–35 bp) of perfect homology to the selected sequence.

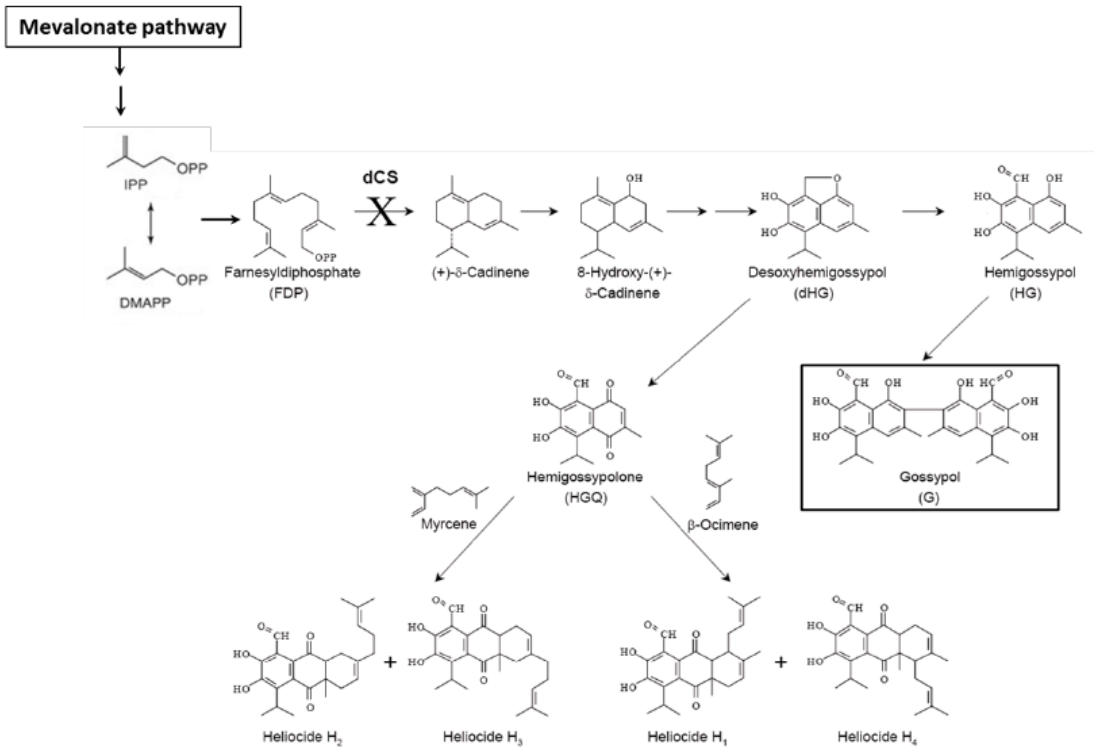

Figure 5. RNAi construct of a *dCS* gene blocks an early step in the biosynthetic pathway for gossypol and related terpenoids in cotton (Figure 3-1 of Texas A&M 2017 petition, the pathway was modified from (Cai et al. 2010)).

The AGP promoter that drives the expression of *dCS* RNAi cassette is a seed-specific promoter, and its seed-specificity in silencing *dCS* genes in cottonseed but not in other plant parts was first confirmed through the analysis of fluorometric β-glucuronidase (*gus*) reporter gene (Appendix A, Texas A&M 2017). The seed-specificity of *dCS* gene suppression was also demonstrated in TAM66274 cotton. The analyses of *dCS* transcript levels, as measured by the quantification cycle (Cq) values of qRT-PCR, in developing seed embryos of TAM66274 cotton and parental variety cv. Coker 312 under field conditions showed that TAM66274 cottonseed embryos had an 86% reduction at 31 days post anthesis (dpa) compared to *dCS* transcript levels in the parental variety cv. Coker 312. In contrast, the *dCS* transcript levels in non-seed tissues, including root, leaf, bract, floral bud, and axillary bud tissues had no significant differences between TAM66274 cotton and cv. Coker 312 (Appendix D, Texas A&M 2017). Thus, the significant reduction (86%) of *dCS* gene transcript levels in the developing cottonseed embryo of TAM66274 cotton but no difference in non-seed tissues is consistent with approximately 97% reduction of gossypol levels in mature cottonseed but equivalent levels of gossypol in non-seed tissues of TAM66274 compared to parental variety cv. Coker 312 (pp.114-116, Texas A&M 2017). These results showed the efficacy of the *dCS* RNAi cassette in selectively suppressing *dCS* transcript levels in embryos, with no effect on transcript levels in other plant parts.

Texas A&M evaluated the potential safety of *dCS* RNAi by using a broad weight of evidence. The RNAi-mediated gene expression regulation is a natural process in eukaryotic organisms, and FDA does not anticipate that the transferred nucleic acids themselves would raise a safety concern as a common food component since nucleic acids are present in the cells of every living organism (FDA 1992). Additionally, RNAi-mediated gene expression suppression has been used in a number of GE crops that have previously been deregulated by USDA and other regulatory authorities, including papaya, potato, plum, common bean, and squash, tomato, and soybean (Parrott et al. 2010). Thus, there is a history of safe consumption of plant products that involve RNAi-mediated gene suppressions. Furthermore, to investigate if *dCS* RNAi would have non-target or adverse effects on human and animals, Texas A&M conducted a BlastN search of the *dCS* RNAi trigger sequence against human, cow, pig, chicken, fish, shrimp, dog and cat expressed sequence tag (EST) sequences. The analyses did not show homology in any 20 base pairs of contiguous stretch of nucleic acids (Appendix D, Texas A&M 2017), supporting that *dCS* RNAi is unlikely to have any adverse non-target effects on human and animals.

Texas A&M also assessed the potential impact of RNAi-mediated suppression of dCS enzyme levels in cotton on other biosynthetic pathways. FDP is a common precursor for many primary and secondary plant metabolites, and only a minute portion of FDP is channeled into gossypol biosynthesis in the glanded cotton plant (Texas A&M 2017). When channeling of FDP into the gossypol pathway is blocked by RNAi silencing of the *dCS* gene, this minute amount of FDP should become available for the production of the primary and secondary plant metabolites through other biosynthesis pathways. These FDP-derived plant metabolites are important cell components and are involved in various aspects of plant growth and development. According to the petitioner's data, which will be discussed later in this document, TAM66274 cotton is equivalent to parental variety cv. Coker 312 in their phenotypic and agronomic characteristics and seed compositions except the intended reduction of gossypol levels in cottonseed, suggesting that silencing of the *dCS* genes in TAM66274 cotton is unlikely to make a significant impact on other biosynthetic pathways associated with the dCS enzyme substrate, FDP.

NPTII Variant Protein

NPTII variant protein expressed in TAM66274 cotton is 273 amino acids in length, and the first 17 amino acids at N-terminal end are derived from *Agrobacterium* NOS protein as a replacement of the first eight amino acids of the native NPTII protein. The NPTII variant protein exhibits the same characteristics as the NPTII protein expressed in other commercial transgenic crops in terms of its resistance to kanamycin and its immune reaction to antibodies specific to NPTII protein. The NPTII variant protein is the same protein expressed in previous deregulated ringspot virus resistant papaya, which has a 19-year history of safe use in food and the environment.

Expression levels of the NPTII variant protein were measured by ELISA in leaf, root, pollen and seed tissues of TAM66274 cotton and parental variety cv. Coker 312 under field conditions. As expected, the protein was not detected in any tissues of the parental variety cv. Coker 312. NPTII variant protein levels in TAM66274 cotton were highest in

leaves (253.3 ng/g dried weight (DW)), significantly lower in roots and seeds (58.5 ng/g and 41.1 ng/g DW, respectively), and undetected in pollen at the detectable level of 25 ng/g DW. NPTII variant protein expression levels in TAM66274 cotton are significantly lower than that in previously deregulated cotton events Bollgard® and Roundup Ready®, i.e. approximately 100-fold and 1000-fold less in leaves and approximately 50-fold and 100-fold less in cottonseed than Bollgard® and Roundup Ready®, respectively. Generally, the amount of NPTII protein expressed in transgenic plants is low, ranging from approximately 0.00005 to 0.001% FW of cottonseed, potato tuber or tomato fruit (Miki and McHugh 2004). The NPTII variant protein amount in TAM66274 cotton cottonseed is extremely low with no more than 0.0000041%.

The *nptII* gene is the most frequently used selectable marker gene for generating transgenic plants and it is found in many of the crops currently approved for commercial production, including corn, potato, oilseed crop, tomato, papaya, cotton, flax, and chicory (Miki and McHugh 2004). The food, feed and environmental safety of the NPTII protein has been evaluated extensively in both the peer-reviewed literature and by regulatory authorities of different countries, and there have been no reports of adverse effects of either NPTII protein or the *nptII* gene on humans, animals or the environment (Miki and McHugh 2004; USDA-APHIS-BRS 2017). Nevertheless, Texas A&M conducted bioinformatic searches of the NPTII variant protein against all known protein allergens and toxins, and confirmed that the NPTII variant protein does not share sequence homology to known protein allergens and toxins.

Potential new ORFs

In addition to *dCS* RNAi and NPTII variant protein, Texas A&M analyzed the potential new open reading frames (ORFs) that are likely to result from the insertion of T-DNA. The ORF search identified a total of 33 putative ORFs of at least 30 amino acids in length in the T-DNA and genomic flanking sequences of TAM66274 cotton, but no ORF was found to span the junction between the TAM66274 cotton genome and the RB or LB regions of the T-DNA insert (Tables 5-4 and 5-5, Texas A&M 2017). Of the above 33 putative ORFs, only six are at least 80 amino acids in length, which is the recommended length of ORFs by the Codex Alimentarius Commission guidelines for potential homology search to known allergens and toxins. Putative ORFs were subjected to bioinformatics analysis for homology to allergens and toxins, and the results showed that all putative ORFs in TAM66274 cotton have no significant similarities to known or putative allergens and toxins, supporting that the putative ORFs in TAM66274 cotton are not potential allergens or protein toxins.

Metabolism composition Analysis

To assess any potential metabolite alteration as a result of the expression of the above inserted genes, Texas A&M analyzed the metabolism composition of TAM66274 cotton grown at eight field sites representing the major cotton-growing regions in the United States (three and five sites during 2014 summer 2015 summer, respectively), in comparison to parental variety cv. Coker 312, and to published values for other commercial cotton varieties (pp.106-139 and Appendix E, Texas A&M 2017). The

purpose of the metabolism composition was to assess the intended effect of reducing cottonseed gossypol levels in TAM66274 cotton and to evaluate if the nutrient and anti-nutrient levels in cottonseed of TAM66274 cotton are compositionally equivalent to parental variety cv. Coker 312 and other conventional cotton varieties. The compositional analyses included proximates (moisture, protein, total fat, ash, carbohydrates and calorie Content), fiber (total dietary, crude, acid and neutral detergent fibers), fatty acids, amino acids, minerals (Cu, Fe, Mn, Zn, Ca, Mg, P, K, and Na), alpha-tocopherol, antinutrients (free and bound gossypol, gossypol isomers, total gossypol, cyclopropenoid fatty acids and phytic acid), and mycotoxin levels (aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2) in cotton seed (Appendix E, Texas A&M 2017).

Based on the accurate and precise HPLC method of measurement, total gossypol levels in TAM66274 cottonseed were less than 370 ppm on a DW basis compared to levels of over 10,000 ppm in cottonseed of parental variety cv. Coker 312 harvested from the same field trials. Total gossypol levels in cottonseed of TAM66274 cotton harvested from 2014 and 2015 field trials were 3.61% and 3.00% of that in parental variety cv. Coker 312, respectively. Therefore, the gossypol levels in cottonseed of TAM66274 cotton were below the maximum allowable level of 450 ppm, considered safe for modified cottonseed products in foods for human consumption (FDA 1960) and below 400 ppm allowed in animal feed (AAFCO 1968). In addition, the ultra-low gossypol cottonseed (ULGCS) trait did not have any meaningful effect on the relative levels of free and bound gossypol, as well as the gossypol isomers (+ and - versions) in the cottonseed.

Other than the above intended reduction in gossypol levels in TAM66274 cottonseed, compositional analyses demonstrated that TAM66274 cottonseed is compositionally and nutritionally equivalent to parental variety cv. Coker 312. It is noted that while majority of the assayed compositional components did not show significant differences between TAM66274 cotton and parental variety cv. Coker 312 across multiple locations during both 2014 and 2015 growing seasons, significant differences in amounts of some individual compositional constituents were detected between the two. For example, statistically significant differences were observed for levels of total dietary fiber, acid detergent fiber, neutral detergent fiber, and alpha-tocopherol in cottonseed during both 2014 and 2015 growing seasons; for cottonseed content of moisture, total fat, carbohydrates, calories, cysteine and tryptophan in 2015 field trials; and for levels of cyclopropenoid fatty acids in 2014 growing season (pp. 106-139, Texas A&M 2017). However, given that the mean and ranges of these analyte values for TAM66274 cotton were within the range of values published in the International Life Sciences Institute (ILSI) crop composition database and/or in the published literature for conventional cotton varieties, these instances of differences in analyte levels between TAM66274 cotton and parental variety cv. Coker 312 were considered due to normal variations in cultivated cotton varieties, and such variations unlikely cause any adverse plant pest and non-target impacts. In other words, these differences were most likely due to small genetic differences between TAM66274 cotton and parental variety cv. Coker 312 resulting from the inherent genetic heterogeneity of the recipient parental population.

The mycotoxin analyses showed that mycotoxin levels, including aflatoxins G1, G2, B1 and B2, as well as deoxynivalenol, acetyldeoxynivalenol, and zearalenone, in cottonseed of TAM66274 cotton are comparable to parental variety cv. Coker 312. Therefore, the intended ULGCS trait did not appear to alter the susceptibility of TAM66274 cottonseed to mycotoxins relative to non-transgenic cv. Coker 312.

In summary, the expression of the inserted DNAs and the resulting phenotype in TAM66274 cotton are consistent with the stability/inheritance of the introduced genetic material. The ORF analysis showed no evidence supporting any potential creation of new ORFs or any unintended effects resulting from the insertion of the genetic materials (Texas A&M 2017). The compositional analyses demonstrated that introduction of *dCS* RNAi construct in TAM66274 cotton achieved the intended reduction in seed gossypol levels while maintaining the equivalent nutrient composition of cottonseed in comparison to cottonseed of parental variety cv. Coker 312, as well as other conventional cotton varieties. There are no observed or anticipated unintended metabolic composition changes in the TAM66274 cotton that could impart any new plant pest or disease risk than non-GE cotton. The previous citations and deregulated petitions for similar genes and gene products, that have a history of safe use and have not been implicated in disease or pest issues, also support that the expression of *nptII* variant gene in TAM66274 cotton are not expected to incur any additional plant pest or increased disease risks.

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences, from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in TAM66274 cotton that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as identified from the previous section). APHIS also assessed whether TAM66274 cotton is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

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, nematode or weed programs exist (USDA-PPQ 2017). Nearly all 17 cotton production states have local regulations concerning the boll weevil (*Anthonomus grandis*), and four states, including Arizona, California, New Mexico, and Texas are quarantined to prevent the spread of the pink bollworm (*Pectinophora gossypiella* (Saund.)) (USDA-PPQ 2017). Eradication updates as of 2014 for both boll weevil and pink bollworm are shown in Figures 6 and 7, respectively. Additionally, as of 2014 USDA APHIS declared the eradication of the cotton seed bug (*Oxycarenus hyalinipennis*) in Florida (USDA-PPQ 2014c).

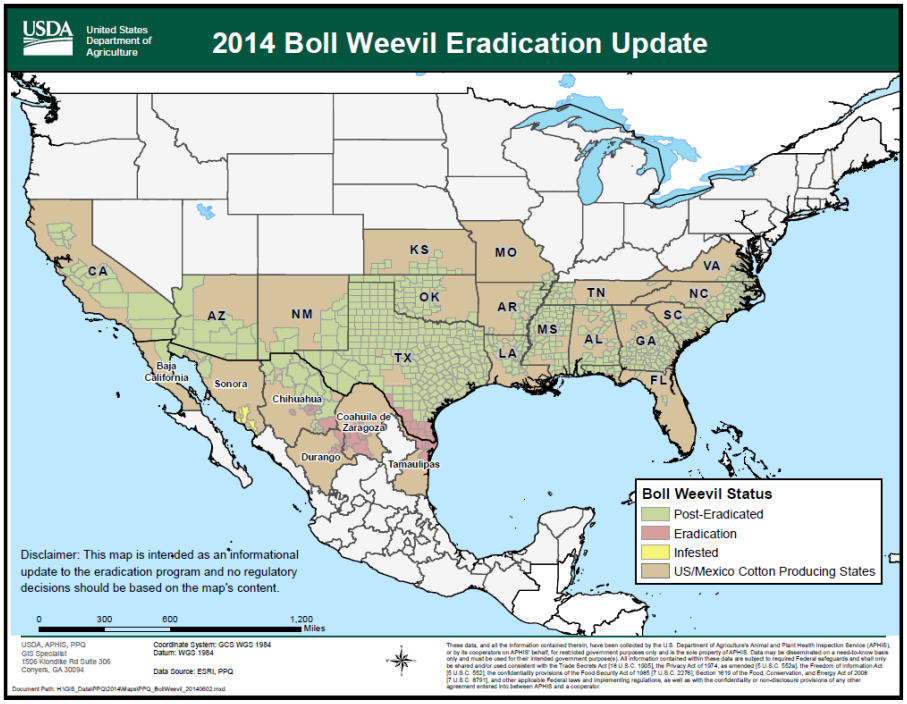

Figure 6. 2014 boll weevil eradication update (USDA-PPQ 2014b)

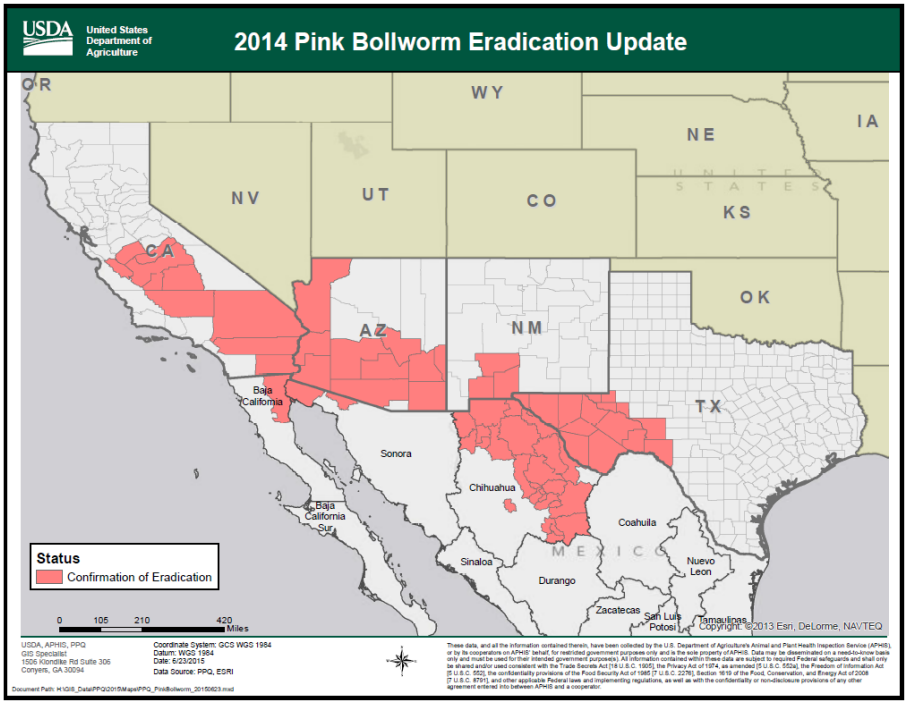

Figure 7. 2014 Pink bollworm eradication update (USDA-PPQ 2014a)

In addition to the above cotton pests, there exist a number of other pests and disease pathogens that infest cotton. Of the various cotton insects, lygus bugs (*Lygus Hesperus, Lygus lineolaris*, and *Lygus elisus*), stink bugs (*Pentatomidae*), thrips (*Thripidae*), bollworm/budworm (*Helicoverpa zea* and *Heliothis virescens*), fall armyworm (*Spodoptera frugiperda*), cotton fleahopper (*Pseudatomoscelis seriatus*), silverleaf whitefly (*Bemisia tabaci*), clouded plant bug (*Neurocolpus nubilus*), and aphids (*Aphididae*) are the most destructive pests based on 2016 U.S. cotton loss data (MSU 2016). Of the various cotton diseases, nematodes (primarily *Meloidogyne spp.* and *Reniform reniformis*), boll rots (*Rhizopus*), leaf spots and others, seedling diseases (primarily *Rhizoctonia solani*, *Pythium* spp., *Phoma exigua*, and *Fusarium* spp.), Verticillium Wilt (*V. dahliae*), and Fusarium Wilt (*F. vasinfectum*) are the most plant-damaging disease pathogens (National Cotton Council 2016).

Cotton itself is not a plant pest in the United States (7 CFR 340; (USDA-NRCS 2017). The Agrobacterium strain *A. tumefaciens* used in the generation of TAM66274 cotton were disarmed and also were already killed with antibiotics during the transformation process (pp. 39-40, Texas A&M 2017). The inserted DNA elements derived from plant pests do not result in the production of infectious agents or disease symptoms in plants. The genetic modifications of TAM66274 cotton, including genetic elements, expression of the gene products and their functions have been summarized above and are not expected to impart any new plant pest or disease risk than non-GE cotton.

Gossypol in cottonseed has significant insecticidal and antimicrobial properties to protect the plant from insects and disease (Stipanovic et al. 1975; Bell and Stipanovic 1978; Stipanovic et al. 1999). In contrast, in naturally occurring glandless mutant cotton

varieties that do not produce gossypol in any plant tissues, the lack of gossypol renders the plant susceptible to insect predation and plant diseases, causing the loss of commercial utility (Bottger et al. 1964; Jenkins et al. 1967; Benedict et al. 1977). This glandless mutant cotton demonstrates the importance of maintaining the protective gossypol and related terpenoids in the vegetative and floral parts of the plant.

Texas A&M genetically engineered TAM66274 cotton to reduce gossypol production selectively in cottonseeds, while leaving gossypol levels unchanged in non-seed tissues (e.g., roots, stems, leaves) where gossypols retain their disease and pest resistance activities (Rathore et al. 2012; Palle et al. 2013). To investigate whether such genetic modifications will alter cotton plant's susceptibility to disease and insect pressure and rodent feeding compared to parental variety cv. Coker 312, TAM66274 cotton and cv. Coker 312 were evaluated under field conditions at eight locations representative of major U.S. cotton production region during the 2014 and 2015 growing seasons (Appendix F, Texas A&M 2017). Plant diseases and insect pests observed in these field studies were typical of those found in commercial cotton cultivation, including plant diseases: leaf spot (*Alternaria* spp., *Cercospora* spp., *Stemphyllium* spp., *Colletrotrichum* spp.) and boll rot (*Fusarium* spp., *Diplodia* spp., *Glomerella gossypii*, *Xanthomonas* spp., *Rhizoctonia* spp., *Alternaria* spp.); insects: thrips (*Frankiella fusca*), tarnished plant bug (*Lygus lineolaris*), stinkbug (*Halyomorpha halys*), cotton bollworm (*Helicoverpa armigera*), and spider mites (*Tetranychidae* spp.). Plant susceptibility to disease and insect pressure was evaluated at 14, 28, 56, 84, and 112 days after planting (DAP). In both 2014 and 2015 field studies, no statistically significant differences in plant disease susceptibility and insect damage were observed in TAM66274 cotton compared to parental variety cv. Coker 312 either across locations or in any individual location (Tables 7-10, 7-11, F-16, and F-17, Texas A&M 2017). Also, no rodent feeding was observed in TAM66274 or parental variety cv. Coker 312 at any field location in both 2014 and 2015 field studies (Tables 7-12 and F-18, Texas A&M 2017). These results demonstrate that the selective reduction of gossypol levels only in cottonseed of TAM66274 cotton did not change plant disease susceptibility and insect damage as well to rodent feeding compared to parental variety cv. Coker 312.

In addition to plant susceptibility to diseases, insect pests and rodents, other phenotypic, agronomic and ecological characteristics of TAM66274 cotton were evaluated at six in-season time points and at harvest under field conditions at eight field sites representative of major U.S. cotton growing regions during the 2014 and 2015 cotton growing seasons. These characteristics include: 1) seed germination, dormancy, and stand count; 2) vegetative growth; 3) reproductive development; 4) fiber quality and 5) plant mapping. Based on the collected data, TAM66274 cotton showed no differences, that would raise concerns with respect to plant pest risk, in phenotypic, agronomic and ecological characteristics compared to the control variety Coker 312, and is comparable to other conventional cotton varieties. Also, as described above in Section C, there are no observed unintended metabolic composition changes in the TAM66274 cotton that could pose any new plant pest or disease risk compared to conventional cotton varieties.

Overall, all the above analyses demonstrate that TAM66274 cotton is unlikely to differ from conventional cotton in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest and disease effects on other agricultural products. For this reason, the cultivation of TAM66274 cotton poses no greater risk of plant pest and disease characteristics compared to the cultivation of control cv. Coker 312 or other conventional varieties.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

TAM66274 cotton is not engineered for pest resistance other than for improving cottonseed quality through the reduction of gossypol levels, thus, there are no 'target' species for this GE cotton. APHIS assessed whether exposure or consumption of TAM66274 cotton would have a direct or indirect adverse impact on nontarget species beneficial to agriculture. Organisms considered were representatives of the species associated with production of the regulated crop in the agricultural environment. The assessment includes an analysis of data and information on TAM66274 cotton compared to the non-GE counterpart Coker 312 for any relevant changes in the phenotype or substances (e.g. proteins, nutrients, or anti-nutrients) produced which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

As described above in Section C "*Expression of inserted DNA, changes in gene expression, new proteins or metabolism*", none of the inserted genetic material in TAM66274 cotton is toxic or produces toxic substances. As mentioned previously in this document, the *nptII* variant gene product is similar in function to the product of *nptII* gene. *nptII* gene and its products were previously evaluated, and no identifiable human and environmental safety issues for the use of *nptII* gene in genetically engineered plants and plant products were identified (Nap et al. 1992; Miki and McHugh 2004; USDA-APHIS-BRS 2017). With respect to *dCS* RNAi gene cassette, the trigger sequences are highly specific only to *dCS* genes in cotton, and thus, will not cause the suppression of genes in other plant or animal species. Furthermore, the use of a highly seed-specific promoter for the RNAi-mediated silencing of *dCS* genes, the reduction of gossypol levels occurs only in cottonseed but not in other plant parts (Table A-3, Texas A&M 2017). This selective reduction of gossypol content only in seeds provides two benefits: i) it makes cottonseed safe for use as feed for various monogastric animals and as human food by reducing or eliminating toxic effect of gossypol on monogastric animals including humans (EFSA 2009; Gadelha et al. 2014); ii) the unaltered gossypol levels in nonseed tissues make cotton plants not more susceptible to insects and disease damages as gossypol plays an important role in the cotton plant's defense against insect pests and diseases.

Texas A&M showed that, other than the improved safety level in terms of gossypol in cottonseed, the seed of TAM66274 cotton is compositionally equivalent to parental variety cv. Coker 312 and other conventional cotton varieties in its proximates, fiber, fatty acids, amino acids, minerals, alpha-tocophenol, phytic acid, and mycotoxins

(pp.106-139, Texas A&M 2017). Field trial-based phenotypic, agronomic and ecological data between TAM66274 cotton and cv. Coker 312 in the responses to biotic or abiotic stressors did not show differences that could impact beneficial organisms associated with the plants (pp.140-166, Texas A&M 2017). Thus, the TAM66274 cotton does not show a detrimental effect on beneficial arthropods compared to the control variety Coker 312.

Therefore, based on the above analysis of the composition of seed and forage tissues, the effect on beneficial arthropods, and the absence of human and environmental safety issues for NPTII protein, APHIS concludes that exposure to and/or consumption of the GE plant are unlikely to have any adverse impacts to non-target organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of TAM66274 Cotton

APHIS assessed whether the GE crop event is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of the GE crop event compared to the nontrangsenic progenitor or the other reference varieties evaluated under field (and/or lab) conditions characteristic for the regions of the U.S. where TAM66274 cotton is intended to be grown. The characteristics for the evaluation of the GE crop event are related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For this crop, such characteristics include seed dormancy and germination, agronomic and phenotypic traits, disease and pest susceptibility, and ecological characteristics.

The assessment also considers whether the engineered trait affects methods of control for the crop in situations where it is managed as a weed or volunteer in subsequent crops or in feral populations.

In the U.S., cotton is not listed as a noxious weed species by the federal government (USDA-NRCS 2017) nor is listed as a weed in the major weed references (7 CFR part 360; (Randall 2017). Further, cotton does not possess weedy characteristics. Cotton is sensitive to low temperature, as temperatures below 15°C or above 38°C will cause growth arrest and freezing temperature even kill cotton plants (OECD 2008; Freeland et al. 2010). The complete growth cycle of cotton generally requires above 15°C for more than 150 days. The low-temperature sensitivity of cotton restricts its geological distribution. Cultivated cotton rarely displays any dormancy characteristics, but may grow as a volunteer under favorable conditions (OECD 2008). If any seeds remain viable and germinate the following year, mechanical methods or herbicides can be used to control volunteers.

The introduction of both the ULGCS and kanamycin resistance traits into cotton is not expected to alter the weediness characteristics of cotton. The ecological impact of the use

of the *nptII* gene in crops has been extensively reviewed and concluded that kanamycin resistance will not lead to enhanced weediness of a *nptII* gene-expressing plant (Nap et al. 1992; Miki and McHugh 2004). Texas A&M conducted field trials at eight sites representative of major cotton production conditions in the U.S. during 2014 and 2015 growing seasons to evaluate phenotypic, agronomic and ecological characteristics comparing TAM66274 cotton with the nontransgenic progenitor (Texas A&M 2017). Agronomic characteristics of TAM66274 cotton related to weediness, such as germination, emergence, seedling vigor, and response to environmental conditions have been shown to be substantially equivalent to parental variety cv. Coker 312. In the few instances where TAM66274 cotton and parental variety cv. Coker 312 showed statistically significant differences such as in plant height, percent of seed germination, and number of seeds per boll, these differences were inconsistent over the two field trial seasons and, therefore, were most likely due to the environmental differences between years and locations (Tables 7-1, 7-6, and 7-7, Texas A&M 2017). However, fiber length of TAM66274 was consistently shorter than the parental variety cv. Coker 312, but within commercially acceptable limits and does not pose a risk of increased weediness or plant pest characteristics. Furthermore, extensive post-harvest monitoring of field trial plots planted with the TAM66274 under USDA-APHIS notifications (Texas A&M 2017) did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. Therefore, these results show that the engineered traits do not confer phenotypic or ecological characteristics resulting in a selective advantage in terms of better survival and reproduction for TAM66274 cotton over the parental control Coker 312. Consequently, TAM66274 cotton is no more likely to become a weed than conventional varieties of the crop.

G. Potential Impacts on the Weediness of Any Other Plants with which TAM66274 Cotton Can Interbreed

Gene flow 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 (Grant 1981); (Riesberg and Wendel 1993; Soltis et al. 1993; Hegde et al. 2006). Even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (Ellstrand 1999). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the GE crop event to sexually compatible relatives in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in the engineered plants.

Potential for gene flow, hybridization and gene introgression

The reproductive biology and pollination characteristics of cotton are well known and have previously been described (OECD 2008). Cotton has complete flower and is

predominately self-pollinating. However, spontaneous cross-pollination can also occur at generally low levels in the presence of suitable insect pollinators (McGregor 1976; Van Deynze et al. 2005; OECD 2008). Normally, the occurrence of transgene introgression through pollen dispersal depends on several factors such as genetic compatibility, mode of pollination, sympatric distribution, etc. A few prerequisites for successful gene flow are: i) presence of sexually compatible relatives; ii) sympatric distribution of GE and its sexually compatible taxa; and iii) overlapping phenology between GE and its sexual compatible taxa (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 (OECD 2008). In addition, transgene introgression will decrease with increasing geographic distance between the source and receiver populations and physical barriers (McGregor 1976; Umbeck et al. 1991; Van Deynze et al. 2005; Zhang et al. 2005; OECD 2008). Field studies demonstrate that upland cotton outcrossing rate declines exponentially with distance from the pollen source, typically below 1% beyond 10 meters (Van Deynze et al., 2005).

As described above in Section B, the genus *Gossypium* consists of 50 species, including four cultivated species, two allotetraploids - *G. hirsutum* and *G. barbadense* (2n = 4x = 52) and the two diploids - *G. arboretum* and *G. herbaceum* (2n = 2x = 26) (OECD 2008; Wendel et al. 2010). In United States and its territories, there exist only two domesticated allotetraploid species, *G. hirsutum* (upland cotton) and *G. barbadense* (Pima or Egyptian cotton), and two wild species, *G. thurberi* (2n = 2X = 26) and *G. tomentosum* (2n = 4X = 52) (USDA-NASS 2017). The two wild species are native to the United States. *G. thurberi* and *G. tomentosum* grow in Arizona and Hawaii, respectively (USDA-NRCS 2017). Upland cotton is sexually compatible with the tetraploids *G. barbadense* and *G. tomentosum*, and can form viable progeny with both species (OECD 2008). Thus, unassisted outcrossing and gene introduction could potentially occur in areas where these species are co-located. However, upland cotton as a tetraploid is effectively incompatible with diploid species such as *G. thurberi*., and they cannot normally hybridize in natural settings and produce fertile offspring (OECD 2008).

Native or naturalized populations[3] of *G. hirsutum* grow in Florida, Puerto Rico, and the Virgin Islands, while naturalized populations grow in some of the Hawaiian Islands (Fryxell 1979; Coile and Garland 2003; Wagner et al. 2012; USDA-NRCS 2014; Lee and Fang 2015; USDA-NRCS 2017; Wunderlin et al. 2017). However, due to eradication efforts to control pink bollworm, native and feral populations of *G. hirsutum* have become very rare in the major U.S. cotton growing areas, and it has even been listed as endangered by the state of Florida (USDA-FS 2013). In Florida, the naturalized populations of *G. hirsutum* are separated by over 120 miles from the nearest commercial cotton production areas in the Florida panhandle (Calhoun County, FL) (Wunderlin et al. 2017; USDA-NASS 2017d). Additionally, in Puerto Rico and the Virgin Islands, there

[3] A "native" plant is one that has grown in a particular region or ecosystem for hundreds or thousands of years. A "naturalized" plant is one that does not need human help to reproduce and maintain itself over time in an area where it is not native USDA-NRCS. 2014. Plants Database: *Native, Invasive, and Other Plant-Related Definitions.* Retrieved from http://plants.usda.gov Last accessed 09/07/2017.

are no commercial cultivation of cotton. Thus, outcrossing from TAM66274 to naturalized *G. hirsutum* is highly unlikely.

G. barbadense is cultivated in many areas where *G. hirsutum* is also grown (USDA-NASS 2017b). Naturalized populations of *G. barbadense* grow in Puerto Rico, the Virgin Islands and most of the Hawaiian Islands, but it is no longer widely grown as an agricultural commodity in Hawaii (Wagner et al. 1990; Pleasants and Wendel 2010; USDA-NRCS 2014, 2017). Although both *G. barbadense* and *G. hirsutum* are predominantly self-pollinating, cross-pollination via insect pollinators can occur both within and between the species (Brubaker et al. 1993; Van Deynze et al. 2005; Llewellyn et al. 2007; OECD 2008).

It has been reported that there is an asymmetrical gene flow between *G. hirsutum* and *G. barbadense*. For example, relatively little gene introgression were found from *G. hirsutum* (male parent) into native or naturalized *G. barbadense* (female parent) in Central America and the Caribbean (Fryxell 1979), while introgression in the reverse direction i.e. from *G. barbadense* (male parent) to native or naturalized *G. hirsutum* (female parent) were found to be relatively common (Wendel et al. 1992; Brubaker et al. 1993). This asymmetry in gene flow between native or naturalized *G. hirsutum* and *G. barbadense*, and the lack of commercial cotton production in Hawaii, Puerto Rico and the Virgin Islands suggest that gene introgression from cultivated TAM66274 cotton to native or naturalized *G. barbadense* is highly unlikely.

The above asymmetric gene flow observed between native and naturalized populations, however, is directionally opposite from gene introgression in modern cultivars of *G. hirsutum* and *G. barbadense*. Specifically, in modern cultivars, the introgression from *G. hirsutum* (male parent) into *G. barbadense* (female parent) is common whereas the introgression from *G. barbadense* (male parent) into *G. hirsutum* (female parent) is rare (Wendel et al. 1992; Brubaker et al. 1993; Van Deynze et al. 2011). Nevertheless, introgression of TAM66274 cotton into *G. barbadense* cultivars is expected to be at the similar low levels observed between cultivated cotton varieties, which also depends on spatial isolation distance and other factors. It is 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 (Van Deynze et al. 2005).

The wild cotton tetraploid species *G. tomentosum* is sexually compatible with upland cotton. However, *G. tomentosum* populations are limited to the Hawaiian Islands. Furthermore, there has been no commercial cotton cultivation in Hawaii, and APHIS has no record showing that seed companies use the Hawaiian Islands as a cotton winter nursery. All these facts suggest that gene introgression from TAM66274 cotton to native populations of *G. tomentosum* is highly unlikely.

In summary, the likelihood of TAM66274 cotton hybridizing with cultivated, wild or feral cotton is low due to the predominance of self-pollination, geographic isolation, and other reproductive barriers. Further, the introduced genetic material in TAM66274

cotton does not cause any major changes in the phenotype of cotton plants other than the intended reduction of gossypol levels in cottonseed. Thus, the engineered traits in TAM66274 cotton is unlikely to cause increased levels of gene flow and introgression from TAM66274 cotton into its sexually compatible relatives. Should outcrossing from TAM66274 cotton to *G. barbadense* or *G. tomentosum* occur, transgene introgression would still require the establishment of hybrid progeny in subsequent generations. In the absence of human aid, the transgenic material in TAM66274 cotton is unlikely to confer a selective advantage on any resulting hybrid progeny.

Potential for enhanced weediness of recipients after gene flow and/or introgression

As discussed above in Section F "*Potential for Enhanced Weediness of TAM66274 Cotton*", the expression of the integrated genetic materials in TAM66274 cotton does not confer or enhance weedy characteristics of cultivated cotton other than to reduce gossypol levels in cottonseed. Should gene flow and/or introgression from TAM66274 cotton to its sexually compatible species occur, the introduced genetic materials are unlikely to cause enhanced weediness of the recipient plants. Thus, APHIS has determined that any adverse consequences of gene flow and/or introgression from TAM66274 cotton to wild relatives or weedy species in the U.S. and its territories are highly unlikely compared to cultivated non-transgenic cotton.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in TAM66274 cotton is not expected to increase the potential for gene flow, hybridization and/or introgression to occur to sexually compatible taxa compared to the nontransgenic recipient or other varieties of the crop commonly grown. Gene flow, hybridization and/or introgression of genes from TAM66274 cotton to other sexually compatible relatives, including wild, weedy, feral or cultivated species in the U.S. and its territories is not likely to occur. Therefore, TAM66274 cotton is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. and its territories.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of the TAM66274 cotton are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

TAM66274 cotton was field-studied in a wide range of environmental conditions in 2014 and 2015 growing seasons in the United States. In eight different sites that represent the major U.S. growing environments, TAM66274 cotton was cultivated and compared with its parental variety cv. Coker 312 under the same agronomic practices (pp. 140-166, Texas A&M 2017). A variety of phenotypic, agronomic and ecological characteristics of TAM66274 cotton were evaluated and compared with the parental variety cv. Coker 312 at six in-season time points and at harvest under field conditions. These characteristics include: 1) seed germination, dormancy, and stand count; 2) vegetative growth; 3)

reproductive development; 4) fiber quality; 5) plant mapping; and 6) plant susceptibility to diseases, insect pests and rodents. Majority of the agronomic and ecological characteristics show no significant differences between TAM66274 cotton and its parental variety cv. Coker 312. In the few characteristics that showed statistically significant between the treatments. However, these differences were inconsistent between the two field trial seasons and, therefore, were not considered agronomically meaningful. The only characteristic that was consistently statistically different between the treatments over the two field trial seasons was fiber length, but the fiber length of TAM66274 cotton was within commercially acceptable range. Therefore, TAM66274 cotton is similar and comparable to conventional cotton varieties in their susceptibility to insects or diseases and as well as their agronomic and ecological characteristics, no changes in cultivation or management practices such as planting times, row spacing, irrigation, crop residue management, tillage or pesticide use are anticipated with the introduction of TAM66274 cotton. Indeed, Texas A&M demonstrated that the cultivation practices needed for growing TAM66274 cotton are essentially the same as used to grow conventional cotton varieties.

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. herbicide and pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of TAM66274 cotton; therefore, no impact on plant diseases or pests or their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which TAM66274 Cotton Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into TAM66274 cotton to be horizontally transferred without sexual reproduction to other organisms 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, pathogens, or parasitic plants. The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields in the biosciences since the late 1940s (Soucy et al. 2015), and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable HGT from genetically engineered organisms to another organism without reproduction or human intervention were reviewed by Keese (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 (Keese 2008; Soucy et al. 2015). HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution (Keese 2008).

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

TAM66274 cotton has six gene elements from bacteria, including the selectable marker gene *nptII* variant from *E. coli*, 51 nucleotides of the 5' end of the nopaline synthase

(*nos*) gene from *A. tumefaciens*, and four non-coding regulatory elements from *A. tumefaciens*, i.e. seven nucleotides of the 25 base pairs of the left border T-DNA repeat, octopine synthase (*ocs)* gene terminator, and *nos* gene promoter and gene terminator.

Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate 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) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (van den Eede et al. 2004; Keeling and Palmer 2008; Keese 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards et al. 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu et al. 2011; Acuna et al. 2012).

Horizontal transfer from plants to bacteria and expression in bacteria of the foreign DNA inserted into the nuclear genome of the GE plant 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* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keeling and Palmer 2008; Keese 2008). Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Brown 2003; Koonin et al. 2011). Third, transgene DNA promoters and coding sequences 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. Fourth, both the FDA (1998) and the European Food Safety Authority (2009) have 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 very rare or remote (FDA 1998; EFSA 2009).

Potential for horizontal gene transfer to viruses

APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. TAM66274 cotton does not contain sequences from plant viruses. Nevertheless, this issue has been considered before by other science review panels and government regulatory bodies (EPA 2006; Keese 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the

point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus) (Frischmuth and Stanley 1998) and RNA viruses which typically replicate in the cytoplasm; however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants and infected related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni et al. 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr et al. 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Recently, a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant (Yoshida et al. 2010). According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS 2013). More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear and 24 –41% of mitochondrial gene transcripts appeared to be acquired from their obligate host species (Xi et al. 2013). However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore in TAM66274 cotton, the DNA

sequences were inserted into the nuclear genome, not the mitochondrial genome (Texas A&M 2017).

If TAM66274 cotton becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from TAM66274 cotton. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis APHIS therefore concludes that HGT of the new genetic material inserted into TAM66274 cotton to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, and other relevant information to assess the plant pest risk of the TAM66274 cotton compared to the unmodified variety from which it was derived. APHIS concludes that the TAM66274 cotton is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in TAM66274 cotton because the *Agrobacterium* (a plant pest) used as a vector to transfer the genetic material was disarmed and was shown not to be present in the final Event TAM66274. The only plant pest sequences in the inserted genetic material are five DNA sequences from *A. tumefaciens*: a 51 nucleotides long fragment of the 5' end region of the nopaline synthase (*nos*) gene and four non-coding regulatory elements, including seven nucleotides of the 25 base pairs of the T-DNA left border repeat, octopine synthase (*ocs)* gene terminator, and *nos* gene promoter and terminator. The addition of these genetic material did not confer any plant pest characteristics to TAM66274 cotton.
- No increase in plant pest risk was identified in TAM66274 cotton from the expression of the inserted genetic material, or changes in metabolism composition because there were no significant changes in agronomic, ecological and compositional characteristics that would render TAM66274 cotton more susceptible to pests and diseases over its control or reference cotton varieties.
- Plant susceptibility to diseases, insect pests and rodents were not observed to be significantly increased or atypical in TAM66274 cotton compared to the nontransgenic counterpart in field trials conducted in growing regions representative of where TAM66274 cotton is expected to be grown. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that TAM66274 cotton is more susceptible to pests or diseases. Therefore, no plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.

- Exposure to and/or consumption of TAM66274 cotton are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of compositional, phenotypic and agronomic data.
- TAM66274 cotton is no more likely to become a weed than conventional cotton varieties based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control TAM66274 cotton as a weed. Volunteers and feral populations of TAM66274 cotton can be managed using a variety of currently available methods and herbicides.
- TAM66274 cotton is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Hybridization and/or introgression of inserted genes from TAM66274 cotton to other sexually compatible relatives with which it can interbreed is not likely to occur. TAM66274 cotton does not confer or enhance weedy characteristics of cultivated cotton.
- Significant changes to agricultural or cultivation practices (e.g. herbicide or pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of TAM66274 cotton were not identified.
- Horizontal gene transfer of the new genetic material inserted into TAM66274 cotton to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

K. References

51 FR 23302. *Coordinated Framework for Regulation of Biotechnology*. Retrieved from regulations.gov

57 FR 22984. *Statement of Policy: Foods Derived from New Plant Varieties*. Retrieved from regulations.gov

AAFCO. 1968. *Cottonseed Products*.

Acuna R, Padilla BE, Florez-Ramos CP, Rubio JD, Herrera JC, Benavides P, Lee SJ, Yeats TH, Egan AN, Doyle JJ, and Rose JK. 2012. *Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee*. PNAS 109, pp. 4197-4202. Retrieved from http://www.pnas.org/content/early/2012/02/17/1121190109.abstract

Barr CM, Neiman M, and Taylor DR. 2005. *Inheritance and recombination of mitochondrial genomes in plants, fungi and animals*. New Phytologist 168, pp. 39-50. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2005.01492.x/abstract

Bell AA and Stipanovic RD. 1978. *Biochemistry of Disease and Pest Resistance in Cotton*. Mycopathologia 65, pp. 91-106.

Benedict JH, Leigh TF, Tingey W, and Hyer AH. 1977. *Gradless Acala Cotton: More susceptible to insects*. California Agriculture, pp. 14-15.

Bottger GT, Sheehan ET, and Lukefahr MJ. 1964. *H.elationof Gossypol Content of Cotton Plants to Insect Resistance*. Journal of Economic Entomology 57, pp. 283-285.

Brown JR. 2003. *Ancient horizontal gene transfer*. Nature Reviews Genetics 4, pp. 121-132. Retrieved from http://www.nature.com/nrg/journal/v4/n2/full/nrg1000.html

Brubaker CL, Koontz JA, and Wendel JF. 1993. *Bidirectional Cytoplasmic and Nuclear Introgression in the New World Cottons, Gossypium barbadense and G. hirsutum (Malvaceae).* American Journal of Botany 80, pp. 1203-1208. Retrieved from http://www.jstor.org/stable/2445549

Cai Y, Xie Y, and Liu J. 2010. *Glandless seed and glanded plant research in cotton. A review*. Agronomy for Sustainable Development 30, pp. 181-190.

Coile N and Garland M. 2003. *Notes on Florida's Endangered and Threatened Plants* (38). Gainesville, FL. Florida Deptartment of Agriculture and Consumer Services.

Dröge M, Pühler A, and Selbitschka W. 1998. *Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern*. Journal of Biotechnology 64, pp. 75–90. Retrieved from http://www.sciencedirect.com/science/article/pii/S0168165698001059

EFSA. 2009. *Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants*. EFSA Journal 1108. Retrieved from http://www.efsa.europa.eu/en/efsajournal/pub/1108.htm

Ellstrand NCP, H.C.; Hancock. 1999. *Gene flow and introgression from domesticated plants into their wild relatives*. Annu. Rev. Ecol. Syst. 30, pp. 539-563.

EPA. 2006. *Plant Incorporated Protectants Based on Virus Coat Protein Genes: Science Issues Asosciated with the Proposed Rule*. Washington, DC. Retrieved from http://www.epa.gov/scipoly/sap/meetings/2005/december/minutes1205.pdf

FDA. 1960. *PARTIALLY DEFATTED, COOKED COTTONSEED FLOUR PERMITTED FOR USE IN FOODS*.

FDA. 1972. *Food Additives Permitted in Food for Human Consumption - COTTONSEED PRODUCTS*.

FDA. 1992. *Statement of Policy: Foods Derived from New Plant Varieties*.

FDA. 1998. *Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants*. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096135.htm

FDA. 2006. *Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use*. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096156.htm Last accessed 21 August 2013.

Freeland TB, Pettigrew B, Thaxton P, and Andrews GL. 2010. Agrometeorology and cotton production. In: *Guide to Agricultural Meterological Practices* (Geneva 2, Switzerland: World Meteorological Organization), pp. 344-352.

Frischmuth T and Stanley J. 1998. *Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus*. Journal of General Virology 79, pp. 1265-1271. Retrieved from http://vir.sgmjournals.org/content/79/5/1265

Fryxell PA. 1979. *The Natural History of the Cotton Tribe (Malvaceae, Tribe Gossypieae)*. Texas A&M University Press, College Station, TX.: Texas A&M University Press.

Fuchs M and Gonsalves D. 2007. *Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies*. Annual Review of Phytopathology 45, pp. 173-202. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.phyto.45.062806.094434?url_ver=Z39.88-2003

Gadelha IC, Fonseca NB, Oloris SC, Melo MM, and Soto-Blanco B. 2014. *Gossypol toxicity from cottonseed products*. TheScientificWorldJournal 2014, pp. 231635. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24895646

Gelvin SB. 2003. *Agrobacterium-Mediated Plant Transformation: the Biology behind the "Gene-Jockeying" Tool*. Microbiology and Molecular Biology Reviews 67, pp. 16-37.

Grant V. 1981. Hybrid Speciation. In: *Plant Speciation* (New York: Columbia University Press), pp. 243-255.

Hegde SG, Nason JD, Clegg JM, and Ellstrand NC. 2006. *The Evolution of California's Wild Radish Has Resulted in the Extinction of Its Progenitors*. Evolution 60, pp. 1187.

Heinstein PF, Herman DL, Tove SB, and Smith FH. 1970. *Biosynthesis of gossypol: incorporation of mevalonate-2-14C and isoprenyl pyrophosphates*. Journal of Biological Chemistry 245, pp. 4658-4665.

Jenkins JN, Maxwell FG, and Parrott WL. 1967. *Field Evaluation of Glanded and Glandless Cotton (Gossypium hirsutum L.) Lines for Boll Weevil (Anthonomus grandis Boh.) Susceptibility*. Crop Science 7, pp. 437-440.

Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T, Sasamoto S, Watanabe A, Idesawa K, Iriguchi M, Kawashima K, Kohara M, Matsumoto M, Shimpo S, Tsuruoka H, Wada T, Yamada M, and Tabata S. 2002. *Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium Bradyrhizobium japonicum USDA110*. DNA Research 9, pp. 189–197. Retrieved from http://dnaresearch.oxfordjournals.org/content/9/6/189.long

Keeling PJ and Palmer JD. 2008. *Horizontal gene transfer in eukaryotic evolution*. Nature Reviews Genetics 9, pp. 605-618. Retrieved from http://www.nature.com/nrg/journal/v9/n8/full/nrg2386.html

Keese P. 2008. *Risks from GMOs due to horizontal gene transfer*. Environmental Biosafety Research 7, pp. 123-149. Retrieved from http://www.ebr-journal.org/action/displayAbstract?fromPage=online&aid=8208895

Khoury CK, Greene S, Wiersema J, Maxted N, Jarvis A, and Struik PC. 2013. *An Inventory of Crop Wild Relatives of the United States*. Crop Science 53, pp. 1496. Retrieved from https://www.crops.org/publications/cs/abstracts/53/4/1496

Koonin EV, Makarova KS, and Aravind L. 2011. *Horizonal Gene Transfer in Prokaryotes: Quantification and Classification*. Annual Review of Microbiology 55, pp. 709-742. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.micro.55.1.709?url_ver=Z39.88-2003

Lee J and Fang D. 2015. Cotton as a World Crop: Origin, History, and Current Status. In: *Cotton, 2nd edition* (Madison, WI: ASA, CSSA, and SSSA), pp. 1-25.

Liu Q, Llewellyn DJ, Singh SP, and Green AG. 2012. Cotton Seed Development: Opportunities to Add Value to a Byproduct of Fiber Produciton. In: *Flowering and Fruiting in Cotton* (Cordova, Tennessee: The Cotton Foundation), pp. 133-162.

Llewellyn D, Tyson C, Constable G, Duggan B, Beale S, and Steel P. 2007. *Containment of regulated genetically modified cotton in the field*. Agriculture, Ecosystems & Environment 121, pp. 419-429. Retrieved from http://www.sciencedirect.com/science/article/pii/S0167880906004543

McGregor SE. 1976. *Insect Pollination Of Cultivated Crop Plants*. USA: United States Deparment of Agriculture Retrieved from https://www.amazon.com/Insect-Pollination-Cultivated-Crop-Plants/dp/B002CIQFZ2

McMichael SC. 1959. *HOPI Cotton, a Source of Cottonseed free of Gossypol Pigments*. Agronomy Journal, pp. 630.

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

Morroni M, Jacquemond M, and Tepfer M. 2013. *Deep sequencing of recombinant virus populations in transgenic and nontransgenic plants infected with Cucumber mosaic virus*. Molecular Plant-Microbe Interactions 26, pp. 801-811. Retrieved from http://apsjournals.apsnet.org/doi/abs/10.1094/MPMI-02-13-0057-R?url_ver=Z39.88-2003

MSU. 2016. *Cotton Insect Losses - 2016*. Retrieved from http://www.entomology.msstate.edu/resources/croplosses/2016loss.asp

Nap J-P, Bijvoet J, and Stiekema WJ. 1992. *Biosafety of kanamycin-resistant transgenic plants*. Transgenic Research 1, pp. 239-249.

National Cotton Council. 2016. Disease Database: *Pest Management*. Retrieved from http://www.cotton.org/tech/pest/ Last accessed August 07, 2017.

OECD. 2008. *Consensus Document on the Biology of Cotton (Gossypium spp.)* (No. 45). Organisation for Economic Co-operation and Development.

OECD. 2009. *CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF COTTON (Gossypium hirsutum and Gossypium barbadense): KEY FOOD AND FEED NUTRIENTS AND ANTI-NUTRIENTS*. Paris, France. Organisation for Economic Co-operation and Development.

OGTR. 2008. *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)*. Australia. Retrieved from http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/biologycotton-toc

Palle SR, Campbell LM, Pandeya D, Puckhaber L, Tollack LK, Marcel S, Sundaram S, Stipanovic RD, Wedegaertner TC, Hinze L, and Rathore KS. 2013. *RNAi-mediated Ultra-low gossypol cottonseed trait: performance of transgenic lines under field conditions*. Plant biotechnology journal 11, pp. 296-304. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23078138

Parrott W, Chassy B, Ligon J, Meyer L, Petrick J, Zhou J, Herman R, Delaney B, and Levine M. 2010. *Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops*. Food and chemical

toxicology : an international journal published for the British Industrial Biological Research Association 48, pp. 1773-1790. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20399824

Pleasants JM and Wendel JF. 2005. *Assessment of potential for gene flow between transgenic cotton and the endemic Hawaiian cotton* (83032901). Ames, Iowa. Iowa State University

Pleasants JM and Wendel JF. 2010. *Reproductive and Pollination Biology of the Endemic Hawaiian Cotton, Gossypium tomentosum (Malvaceae)*. Pacific Science 64, pp. 45-55. Retrieved from http://www.bioone.org/doi/full/10.2984/64.1.045

Randall RP. 2017. *A Global Compendium of Weeds*.

Rathore KS, Sundaram S, Sunilkumar G, Campbell LM, Puckhaber L, Marcel S, Palle SR, Stipanovic RD, and Wedegaertner TC. 2012. *Ultra-low gossypol cottonseed: generational stability of the seed-specific, RNAi-mediated phenotype and resumption of terpenoid profile following seed germination*. Plant biotechnology journal 10, pp. 174-183. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21902797

Richards TA, Soanes DM, Foster PG, Leonard G, Thornton CR, and Talbot NJ. 2009. *Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi*. Plant Cell 21, pp. 1897-1911. Retrieved from http://www.plantcell.org/content/21/7/1897.long

Richardson AO and Palmer JD. 2007. *Horizontal gene transfer in plants*. Journal of Experimental Botany 58, pp. 1-9. Retrieved from http://jxb.oxfordjournals.org/content/58/1/1.long

Riesberg LH and Wendel JF. 1993. Introgression and Its Consequences in Plants. In: *Hybridization Zones and the Evolutionary Process* (New York: Oxford University Press), pp. 70-109.

Soltis DE, Soltis PS, and Rieseberg LH. 1993. *Molecular Data and the Dynamic Nature of Polyploidy*. Critical Reviews in Plant Sciences 12, pp. 243-273.

Soucy SM, Huang J, and Gogarten JP. 2015. *Horizontal gene transfer: building the web of life*. Nature reviews. Genetics 16, pp. 472-482. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26184597

Stace CA. 1987. *Hybridization and the plant species*.

Stipanovic RD, Bell AA, and Benedict CR. 1999. Cotton Pest Resistance: The Role of Pigment Gland Constituents. In: *Biologically Active Natural Products: Agrochemicals* (Washinton, D.C.: CRC Press), pp. 211-217.

Stipanovic RD, Bell AA, Mace ME, and Howell CR. 1975. *Antimicrobial terpenoids of Gossypium 6-methoxygossypol and 6, 6'-dimethoxygossypol*. Phytochemistry 14, pp. 1077-1081.

Sunilkumar G, Campbell LM, Puckhaber L, Stipanovic RD, and Rathore KS. 2006. *Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol*. Proceedings of the National Academy of Sciences of the United States of America 103, pp. 18054-18059. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17110445

Thompson JR and Tepfer M. 2010. *Assessment of the Benefits and Risks for Engineered Virus Resistance*. Advances in Virus Research 76, pp. 33-56. Retrieved from http://www.sciencedirect.com/science/article/pii/S0065352710760024

Turturo C, Friscina A, Gaubert S, Jacquemond M, Thompson JR, and Tepfer M. 2008. *Evaluation of potential risks associated with recombination in transgenic plants expressing viral sequences*. The Journal of General Virology 89, pp. 327-335. Retrieved from http://vir.sgmjournals.org/content/89/1/327.long

Umbeck PF, Barton KA, Nordheim EV, McCarthy JC, Parrott WL, and Jenkins JN. 1991. *Degree of Pollen Dispersal by Insects from a Field Test of Genetically Engineered Cotton*. Journal of Economic Entomology 84, pp. 1943-1950. Retrieved from http://jee.oxfordjournals.org/content/84/6/1943.abstract Last accessed July 20, 2016.

USDA-APHIS-BRS. 2017. *Petitions for Determination of Nonregulated Status*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status

USDA-ERS. 2017. *Cotton Costs and Returns*. United States Department of Agriculture/Economic Research Service. Retrieved from https://www.ers.usda.gov/data-products/commodity-costs-and-returns/commodity-costs-and-returns/#Recent Costs and Returns: Cotton

USDA-FAS. 2017. *Oil Seeds - World MArkets and Trend*. United States Department of Agriculture/Foreign Agricultural Service. Retrieved from https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf

USDA-FS. 2013. *Wildland Shrubs of the United States and Its Territories: Thamnic Descriptions*.

USDA-NASS. 2017. *US Cotton Quick Stats*. Retrieved from https://quickstats.nass.usda.gov/data/printable/917A40FE-7B7C-3D69-BAAC-D9F0A1AE6402

USDA-NASS. 2017a. *Cotton Production Areas*. United States Department of Agriculture/National Agricultural Statistics Service. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/cotnacm.pdf

USDA-NASS. 2017b. *US Cotton Acerage by Year*. United States Department of Agriculture. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/cotnac.pdf

USDA-NASS. 2017c. *US Cotton Yield by Year*. United States Drepartment of Agriculture. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/cotnyld.pdf

USDA-NASS. 2017d. *ArcGis Map of Florida Wild Upland Cotton and Commerical Upland Cotton Production*. Washington, DC.

USDA-NRCS. 2013. *Striga asiatica (L.) Kuntze, Asiatic witchweed*. Retrieved from http://plants.usda.gov/core/profile?symbol=STAS2 Last accessed 21 August 2013.

USDA-NRCS. 2014. Plants Database: *Native, Invasive, and Other Plant-Related Definitions*. Retrieved from http://plants.usda.gov Last accessed 09/07/2017.

USDA-NRCS. 2017. Plants Database: *Cotton Plant Profile*. Retrieved from https://plants.usda.gov/core/profile?symbol=GOHI

USDA-PPQ. 2014a. *2014 Pink Boll Worm Eradication Update*. Washington, DC. Retrieved from

https://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/downloads/pbw-map.pdf
USDA-PPQ. 2014b. *2014 Boll Weevil Eradication Update*. Washington, DC. Retrieved from https://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/downloads/bwe-map.pdf
USDA-PPQ. 2014c. *Cotton seed Bug Eradication*. Retrieved from https://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/downloads/DA-2014-15.pdf
USDA-PPQ. 2017. *Cotton Pest Programs*. USDA-PPQ. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/cotton-pests/ct_cotton_pests_home Last accessed August 04, 2017.
van den Eede G, Aarts H, Buhk HJ, Corthier G, Flint HJ, Hammes W, Jacobsen B, Midtvedt T, van der Vossen J, von Wright A, Wackernagel W, and Wilcks A. 2004. *The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants*. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 42, pp. 1127-1156. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15123384
Van Deynze AE, Sundstrom FJ, and Bradford KJ. 2005. *Pollen-Mediated Gene Flow in California Cotton Depends on Pollinator Activity*. Crop Science 45, pp. 1565. Retrieved from https://dl.sciencesocieties.org/publications/cs/abstracts/45/4/1565
Van Deynze AE, Hutmacher RB, and Bradford KJ. 2011. *Gene Flow between G hirsutum and G barbadense is Asymmetric*. Crop science 51, pp. 298-305.
Wagner WL, Herbst DR, and Sohmer SH. 1990. *Manual of the flowering plants of Hawai'i*. Bishop Museum Special Publication 53.
Wagner WL, Herbst DR, Khan N, and Flynn T. 2012. *Hawaiian vascular plant updates: a supplement to the Manual of the Flowering Plants of Hawai'i and Hawai'i's ferns and fern allies, Version 1.3*. http://www.botany.si.edu/pacificislandbiodiversity/hawaiianflora/supplement.htm
Wendel JF, Brubaker CL, and Percival AE. 1992. *Genetic Diversity in Gossypium hirsutum and the Origin of Upland Cotton*. American Journal of Botany 79, pp. 1291-1310. Retrieved from http://www.jstor.org/stable/2445058?seq=1#page_scan_tab_contents Last accessed 05/11/2014 13:12.
Wendel JF, Brubaker CL, and Seelanan T. 2010. The Origin and Evolution of Gossypium. In: *Physiology of Cotton* (Springer Netherlands), pp. 1-18. Retrieved from http://link.springer.com/chapter/10.1007/978-90-481-3195-2_1
Wood DW, Setubal JC, Kaul R, Monks DE, Kitajima JP, Okura VK, Zhou Y, Chen L, Wood GE, Almeida NF, Jr., Woo L, Chen Y, Paulsen IT, Eisen JA, Karp PD, Bovee D, Sr., Chapman P, Clendenning J, Deatherage G, Gillet W, Grant C, Kutyavin T, Levy R, Li MJ, McClelland E, Palmieri A, Raymond C, Rouse G, Saenphimmachak C, Wu Z, Romero P, Gordon D, Zhang S, Yoo H, Tao Y, Biddle P, Jung M, Krespan W, Perry M, Gordon-Kamm B, Liao L, Kim S, Hendrick C, Zhao ZY, Dolan M, Chumley F, Tingey SV, Tomb JF, Gordon MP,

Olson MV, and Nester EW. 2001. *The genome of the natural genetic engineer Agrobacterium tumefaciens C58*. Science 294, pp. 2317-2323. Retrieved from http://www.sciencemag.org/content/294/5550/2317.long

Wunderlin RP, B. F. Hansen, A. R. Franck, and Essig FB. 2017. PlantAtlas.org: *Atlas of Florida Plants*. Retrieved from http://florida.plantatlas.usf.edu/

Xi Z, Wang Y, Bradley RK, Sugumaran M, Marx CJ, Rest JS, and Davis CC. 2013. *Massive mitochondrial gene transfer in a parasitic flowering plant clade*. PLoS Genetics 9. Retrieved from http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003265

Yoshida S, Maruyama S, Nozaki H, and Shirasu K. 2010. *Horizontal Gene Transfer by the Parasitic Plant Striga hermonthica*. Science 328, pp. 1128.

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

Zhu B, Lou MM, Xie GL, Zhang GQ, Zhou XP, Li B, and Jin GL. 2011. *Horizontal gene transfer in silkworm, Bombyx mori*. BMC genomics 12, pp. 248. Retrieved from http://www.biomedcentral.com/1471-2164/12/248