Plant Pest Risk Assessment for Syngenta COT67B Cotton

Syngenta Biotechnology, Inc., (referred to hereafter as Syngenta) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture for a determination that genetically engineered (GE) cotton (*Gossypium hirsutum*) event COT67B (APHIS number 07-108-01p) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000[1]. This plant pest risk assessment was conducted to determine if COT67B cotton is unlikely to pose a plant pest risk.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of COT67B and its progeny in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if COT67B is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, then APHIS has no regulatory authority over that organism.

Based on the information requested by APHIS for submission of a petition for determination of nonregulated status (§ 340.6(c)) and in order to determine whether COT67B is unlikely to pose a plant pest risk, BRS considered information such as: plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, agricultural or cultivation practices, effects of the regulated article on non-target organisms, and transfer of genetic information to organisms with which it cannot interbreed.

The Environmental Assessment (EA) for this petition included an analysis on agricultural or cultivation practices for COT67B. Potential impacts addressed in this risk assessment are those that pertain to plant pest risk characteristics. The GE construct inserted in COT67B was evaluated to determine if the sequences inserted into the cotton could cause plant disease. In addition, morphological characteristics of this cotton were analyzed to determine if this variety would become weedy or invasive. Gene flow and introgression of the inserted genes into weedy and wild relatives was evaluated to determine the potential of increased weedy or invasive characteristics; also, the potential to transfer genetic information to organisms with which cotton cannot interbreed. Finally, APHIS evaluated and compared COT67B to conventional cotton in regards to disease and pests susceptibility and conducted an analysis of the effects of the regulated article on non-target organisms. A thorough assessment of the effects of the determination on non-

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[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(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."

target and beneficial organisms, and threatened and endangered species was included in the EA.

Development of Lepidopteran Resistant Cotton event COT67B

Cotton growers in the United States, the world's largest exporter of cotton, suffer significant yield loss due to insect pests (NCC 2008). There is a need to provide growers with a cost effective method to control insects, while taking into account the potential hazardous nature of insecticides. GE cotton expressing Cry proteins derived from *Bacillus thuringiensis* (*Bt*), commonly referred to as *Bt* cotton, have a long history of safe use without adverse human health or environmental effects and provide an option for the control of lepidopteran insect pests (Glare 2000, Romeis 2008, NAS 2004).

Syngenta has genetically engineered *G. hirsutum* (referred to as Upland cotton or cotton) to express a Cry1Ab protein for use in the control of lepidopteran pests. COT67B deters feeding by several insects known to cause significant damage to cotton in the U.S. These include: tobacco budworm (*Heliothis virescens*), cotton bollworm (*Helicoverpa zea*), pink bollworm (*Pectinophora gossypiella*), and cabbage looper (*Trichoplusia ni*) (USDA-APHIS 2008, Williams 2008).

GE cotton containing insect-resistant traits, first approved for commercial production in the U.S. in 1996, has been widely adopted by U.S. growers (USDA-AMS 2009, USDA-ERS 2009). Most of the growers who adopt *Bt* cotton do so mainly to increase yields through improved pest control (USDA-ERS 2006). Plantings of GE insect resistant cotton increased rapidly in the U.S. to the current 65 percent (Figure 1) (USDA-ERS 2009). Transgenic varieties - GE varieties resistant to worms, herbicides, or both - accounted for 88 percent of the Upland cotton planted in the U.S. in 2009 (USDA-ERS 2009). Grower benefits are likely to be higher with *Bt* cotton in areas and years with high infestation levels of the target pests (EPA 2008, USDA-ERS 2006).

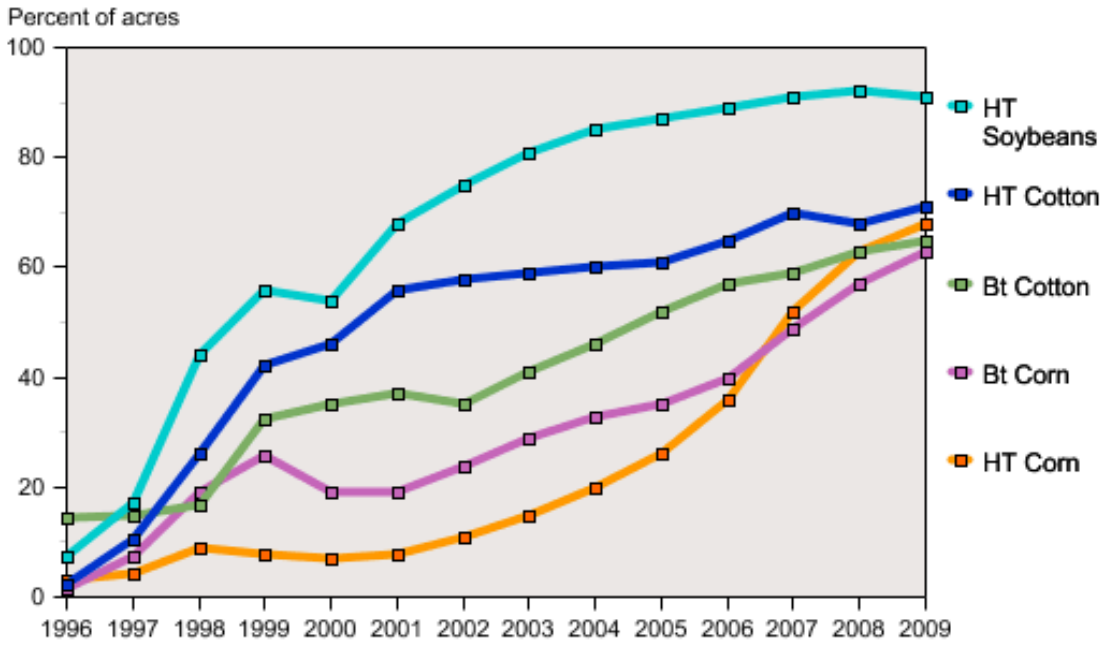

Figure 1. Adoption rates of genetically engineered crops in the U.S. (USDA-ERS 2009).

Tolerance exemptions and conditional pesticide registrations have been granted for the plant-incorporated protectant in COT67B and the genetic material necessary for its production. On July 16, 2008, the Environmental Protection Agency (EPA) granted an exemption from the requirement of a tolerance for residues of Cry1Ab in or on food and feed commodities of cotton (73 FR 40760-40764). Likewise, on October 29, 2008, EPA approved the conditional registration of Cry1Ab produced in COT67B for use as a lepidopteran insecticide (73 FR 64323-64324). Syngenta's food safety summary submitted to FDA indicated that food and feed derived from COT67B are as safe and nutritious as those derived from conventional cotton. Following this voluntary biotechnology consultation, on February 13, 2009, the FDA concluded that it had "no further questions concerning food and feed derived from cotton event COT67B" (FDA BNF No. 0112).

Description of the Modification

COT67B has been genetically engineered to contain one transgene construct[2]: an actin promoter, a full-length *cry1Ab* gene, and a nopaline synthase terminator, and non-coding regulatory regions.

[2] The *aph4* gene from *Escherichi coli*, encoding for the selectable marker hygromycin-B phosphotransferase, was also incorporated as part of the initial transformation process. However, the transformation process resulted in the introduction of the *cry1Ab* gene and the *aph4* gene into two different chromosomes. The presence of the *aph4* gene allowed for selection of transformed cells containing *cry1Ab* gene in the presence of hygromycin. As part of the breeding process to develop COT67B, the *aph4* gene was segregated out, and is not present COT67B (Syngenta 2007, Figures 3-13 through 3-16).

- The actin promoter and intron from the actin-2 gene of *Arabidopsis thaliana* (An 1996).

- The full-length *cry1Ab* gene from *B. thuringiensis* subsp. *kurstaki* strain HD-1 encodes the Cry1Ab protein. COT67B contains a full-length c*ry1Ab* gene (*flcry1Ab*, 3546bp) which encodes a full-length crystal protein comprising 1181 amino acids (Syngenta 2007, Appendix 1-Figure1). Full-length c*ry1Ab* in COT67B was originally derived from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 (*Btk*). The native or wild-type *cry1Ab* gene is a product of the genetic recombination of the *B. thuringiensis kurstaki* HD-1 genes, *cry1Aa* and *cry1Ac*. It is well documented that mutations resulting in deletions of stretches of DNA are common during genetic recombination. Such was the case for the native *cry1Ab* gene (Geiser 1986). This loss of DNA resulted in a diminished capacity of native *cry1Ab* to encode Cry1Ab protein in fermentative cultures of *B. thuringiensis* under the customary fermentation temperatures. It was subsequently discovered that this inefficiency was attributable to the absence of 26 amino acids encoded by the stretch of DNA lost as a result of recombination. Syngenta "repaired" the gene by replacing the deleted coding region with the functional region from *cry1Aa*, thus restoring the original fermentative properties of *cry1Ab*. The additional 26 amino acids in the C-terminal portion of the FLCry1Ab protein are referred to as the 'Geiser motif' (Geiser 1986, 1991; Koziel 1997) and are encoded by the "full-length" cry1Ab (*flcry1Ab)* gene. This "full-length" version of the gene was used in transformation to produce COT67B and is referred to as full-length *cry1Ab*. The Cry1Ab protein is toxic to certain lepidopteran species.

- The terminator sequence was derived from the nopaline synthase gene of the plant pest *A. tumefaciens* (Entrez Accession Number V00087 (NCBI 2007)). Its function is to provide a polyadenylation site [3] (Depicker 1982).

- The right and left border regions of T-DNA from *Agrobacterium tumefaciens* nopaline Ti-plasmid.

Transgenic DNA was introduced into the parental cotton line Coker 312, using disarmed (non-plant pest causing) *Agrobacterium*-mediated transformation. Plant cells containing the introduced DNA were selected by culturing in the presence of hygromycin. After the initial incubation, the broad-spectrum antibiotic cefotaxime was included in the culture medium to kill any remaining *A. tumefaciens*. Because the transformed cells contain sequences from plant pathogens, they are explicitly subject to regulation under 7 CFR part 340.

[3] Polyadenylation site – facilitates the addition of adenine nucleotides to the 3′ end of messenger ribonucleic acid molecules during posttranscriptional modification.

Data from Southern analyses demonstrate that COT67B plants contain: (1) a single copy of *cry1Ab* (Syngenta 2007, Figure 3-8); (2) a single copy of the actin promoter (Syngenta 2007, Figure 3-10); (3) no sequences from the transformation plasmid (pNOV4641) (Syngenta 2007, Figure 3-12) (*i.e.*'backbone sequences'); (4) no sequences from the *aph4* gene. Analysis of DNA sequences of COT67B confirmed that the overall integrity of the intended insert and the contiguousness of the functional elements have been maintained (Syngenta 2007, Appendix 1 - Figure 4). Statistical analyses over multiple generations confirm that the *cry1Ab* gene is stably integrated and is inherited over generations in the expected fashion (Syngenta 2007, Figures 3-17 and 3-18).

Potential of COT67B to Become Invasive and/or a Weed

Cotton is not considered a weed in the U.S. (Holm 1977 and 1997, Muenscher 1980, Reed 1977) and is not listed as a Federal noxious weed (USDA-NRCS 2006). APHIS assessed whether COT67B is any more likely to become a weed than the nontransgenic comparator, or other cultivated cotton. The assessment encompassed a thorough consideration of the basic biology of cotton and an evaluation of the unique characteristics of COT67B. APHIS analyzed the field test reports and other data included in the petition, as well as data from scientific literature on the establishment, reproduction, and dispersal processes of cotton as measures of invasiveness and weediness.

Syngenta provided measures of plant growth including; plant height, days to flowering, total nodes[4], plant height to node ratio, number of bolls per plant and days to open boll (Syngenta 2007, Tables 5-3, 5-8, 5-9, 5-10, 5-16 through 5-19 and Appendix 4). Some statistically significant differences in plant growth characteristics were noted in various field trials. For instance, plant height was similar to both comparators for early square, early bloom and late bloom stage of plant development in 2005 (Syngenta 2007, Tables 5-8, 5-9 and 5-10) and for early square stage in 2006 (Syngenta 2007, Table 5-16). In 2006 the early bloom, late bloom, and preharvest stages of COT67B had similar plant height when compared to its COT67B null isoline[5] (Syngenta 2007, Tables 5-17, 5-18 and 5-19), however, COT67B on average was slightly shorter than the parental Coker 312 (Syngenta 2007, Table 5-17, 5-18 and 5-19). The observed variations in growth were small, inconsistently seen in all stages of plant development. The differences in growth were minor and not found in all growing environments and therefore, not expected to be a factor in terms of weediness or pest potential.

A measure of the reproductive capacity of plants that are propagated by seed, such as cotton, is the number of seeds that are produced and the germination and viability of

[4] A node is the point on the stem from which a leaf grows.

[5] Null isoline refers to a plant line that is genetically very similar to another plant line except for the presence of a different allele at one locus. Because there is only one copy of the *cry1Ab* gene in COT67B, when the COT67B is crossed with a cultivar that lacks the *cry1Ab* gene, 50 percent of the progeny will lack the *cry1Ab* gene. In this case, the null isoline refers to the progeny of the original transformed COT67B that lack the *cry1Ab* gene.

those seeds. Overall, COT67B produced similar or slightly lower levels of seed when compared to Coker 312 or the null isoline (Syngenta 2007, Tables 5-4, 5-11 and 5-20). Seed germination tests showed no significant differences in relative germination (Syngenta 2007, Table 5-12).

These results on growth characteristics and seed production and germination, indicate that the COT67B is not significantly different than its comparators. In addition to the results summarized above, APHIS notes that there have been no reports of increased weediness associated with other lepidopteran insect resistant cotton lines that have been deregulated pursuant to Part 340 and the Plant Protection Act (Carpenter 2002, USDA-APHIS 2009). There is no indication that COT67B possesses a selective advantage that would result in increased weediness. Therefore, COT67B lacks the ability to persist as a troublesome weed, and there would be no direct impact on current weed management practices for cotton cultivation.

Potential for Gene Flow and Gene Introgression from COT67B into its Sexually-Compatible Relatives

G. hirsutum is a perennial plant that is cultivated as an annual in the U.S. where it is grown from Virginia southward and westward to California (McGregor 1976, USDA-NASS 2009). *G. hirsutum* is generally self-pollinating and its pollen is not readily dispersed by the wind because it is sticky and heavy (Khan 1950, Thies 1953). Insect pollination is generally carried out by bumble bees, Melissodes bees, and honey bees (McGregor 1976). In assessing the risk of gene introgression from COT67B to its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression, and 2) the potential impact of introgression.

Although most of the cultivated cotton grown in the U.S. is *G. hirsutum*, *G. barbadense* (Pima cotton) is also grown (USDA-NASS 2009). In addition to these cultivated species, *G. thurberi* and *G. tomentosum* are found in the mountains of southern Arizona and in Hawaii, respectively. None of the above are listed (or proposed) as endangered or threatened under Federal (USFWS 2009a, USFWS 2009b) or State listings (Arizona 2009, Hawaii 2001) with the exception of *G. hirsutum*.

Wild populations of *G. hirsutum* have been listed as threatened and endangered by the State of Florida (Florida 2003). However, in Florida, wild *G. hirsutum* is not present in the northwestern panhandle where cotton cultivation occurs (Coile 2003, USDA-NASS 2009, Wunderlin 2004). Additionally, because the terms and conditions of EPA's conditional registration for Cry1Ab in cotton prohibits commercial cultivation south of Route 60 (near Tampa (EPA 2008)), COT67B is neither expected to be planted commercially in the areas of Florida where wild populations of *G. hirsutum* occur nor would they likely be impacted by COT67B planted north of Route 60. Therefore, because they are not likely to be present in close proximity, cultivated *G. hirsutum* is not likely to cross with wild populations of *G. hirsutum*.

G. tomentosum is native to the Hawaiian Islands, occurring primarily in arid, rock, or clay coastal plains (Wagner 1999). In laboratory and greenhouse breeding programs with hand pollination, *G. tomentosum* and *G. hirsutum* are sexually compatible (Meyer 1974), form viable progeny and share common pollinators (Pleasants 2010). However, DNA marker analyses have not found evidence of genes from *G. hirsutum* occurring in native populations of *G. tomentosum* (DeJoode 1992). It is possible that the lack of evidence of movement of *G. hirsutum* genes into *G. tomentosum* is the result of lack of opportunity, because cotton has not been grown commercially in Hawaii for at least the last 45 years (USDA-NASS 2009). *G. tomentosum* is not known to be weedy or to have invasive characteristics (Holm 1977, Holm 1997, University of Hawaii 2001), and is considered a rare plant in Hawaii (University of Hawaii 2001). Because *G. tomentosum* is not a weedy plant, even if *cry1Ab* did introgress into *G. tomentosum* it is not likely to become weedy or invasive. Finally, EPA's conditional registration for Cry1Ab prohibits commercial use of COT67B in Hawaii (EPA 2008), therefore it would not be planted in Hawaii and the probability of outcrossing would not exist.

The difference in chromosome numbers precludes sexual compatibility of *G. hirsutum* with *G. thurberi* (OECD 2008). Outcrossing of genes from COT67B to *G. thurberi* is unlikely to occur, because *G. thurberi* contains only the D genome whereas *G. hirsutum* contains both the A and D genome. In addition, in Arizona, *G. thurberi* was eradicated near cotton growing areas as part of the cotton boll weevil control program (Benson 1981, Kearney 1960) and the probability of outcrossing would be very unlikely. Therefore, USDA has determined that any adverse consequences of gene flow from COT67B to wild or weedy species in the U.S. are highly unlikely.

Potential for Transfer of Genetic Information to Organisms with which COT67B Cannot Interbreed

Horizontal gene transfer and expression of DNA from a plant species to bacteria is unlikely to occur (Keese 2008). First, many bacteria (or parts thereof) that are closely associated with plants have been sequenced, including *Agrobacterium* and *Rhizobium* (Kaneko 2000, Kaneko 2002, Wood 2001). There is no evidence that these organisms contain genes derived from plants. 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 2001). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not bacterial expression. Thus, even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

Potential for COT67B to have Altered Disease and Pest Susceptibilities

APHIS assessed whether COT67B is likely to have significantly altered disease and pest susceptibility. This assessment encompassed a thorough consideration of the introduced trait and disease and pest susceptibility data from COT67B field trials.

Transformation of *G. hirsutum* with *Agrobacterium* will not lead to crown gall disease, because the *A. tumefaciens* strain GV3101 was disarmed by removing the native T-DNA (Syngenta 2007, page 31, Klee 2000). (The native T-DNA contains the plant hormone genes necessary for the formation of crown gall tumors.) Instead, a T-DNA region that contains a *cry1Ab* gene as well as the regulatory components necessary for their expression in the cotton genome, was introduced on plasmid pNOV4641 (Syngenta 2007, Table 3-1). Further, antibiotics were used to kill any remaining *Agrobacterium* after the transformation.

The DNA inserted into COT67B includes only, the actin promoter from the plant *A. thaliana*, the *cry1Ab* gene from the bacterium *B. thuringiensis*, commonly found in soils, and the nopaline synthase termination sequence from *A. tumefaciens* (see the Description of the Modification in this document). The *A. tumefaciens* nopaline synthase terminator has been well-characterized and cannot cause disease (Hooykaas 1992). The actin promoter sequence is derived from a plant that is not a plant pest and is therefore highly unlikely to make COT67B susceptible to disease and plant pests.

COT67B was grown during 2004, 2005, and 2006, in 22 locations encompassing the range of geographical and environmental conditions where it is anticipated that it will be grown commercially in the U.S. These field trials included two cotton comparators: the COT67B null isoline and Coker 312. COT67B plants were examined for changes in the types of plant pests that were observed and the relative pest damage that those plants incurred (field test reports submitted for COT67B, Syngenta 2007, Tables 1-1 and 5-6). When expressed in COT67B, the Cry1Ab protein provides protection from the feeding damage incurred by several important lepidopteran pests of cotton (Syngenta 2007, Supplements 20 and 22).

Data submitted by Syngenta indicate that there are no significant differences for disease susceptibility between COT67B cotton and its comparators (field test reports for field trials of COT67B submitted to APHIS by Syngenta, Syngenta 2007, Table 5-1). All cotton plants (GE and non-GE) produce the anti-nutrients[6] gossypol and cyclopropenoid fatty acids (OECD 2004). Data presented by Syngenta showed that the levels of both gossypol and cyclopropenoid produced in COT67B cotton fall within normal ranges of commercially available cotton varieties (Syngenta 2007, Table 5-30). Overall, data presented by Syngenta indicate that the composition of COT67B is not significantly different from its non-transgenic comparators except for the presence of the inserted sequence (Syngenta 2007, Tables 5-25 through 5-29 and 5-31). Based upon the field trial and composition data, it appears that COT67B exhibits the same pest susceptibility as conventional cotton, except for the increased resistance to lepidopteran pests.

[6] Antinutrients are substances that inhibit nutrient metabolism or absorption. Gossypol provides defense against herbivory (insect feeding) and microbial attack. Cyclopropenoid fatty acids are known to inhibit the desaturation of stearic acid to oleic acid in animals. Because of the toxic effect of these compounds, maximum allowable limits have been set for levels of these compounds in cotton that is used for food and feed (OECD 2004).

Additionally, it does not appear that COT67B harbors an altered pest or pathogen community compared to other cotton varieties (field test reports submitted by Syngenta for COT67B, Syngenta 2007, Table 5-1).

Potential Impacts of COT67B on Target and Non-target Organisms, Including Beneficial Organisms

For decades, microbial products containing *B. thuringiensis* (the organism that produces the Cry1A protein) have been used to control insect pests on a commercial scale and for home garden applications (Glare 2000, OMRI 2008, Shelton 2002). Cry1Ab proteins display lepidopteran-specific toxic activity against the early-instar larvae of agronomically important insect pests such as pink bollworm (*Pectinophora gossypiella*), tobacco budworm (*Heliothis virescens*), cotton bollworm (*Helicoverpa armigera*), armyworms (*Spodoptera* spp.), European corn borer (*Ostrinia nubilalis*), and diamondback moth (*Plutella xylostella*) (Glare 2000, Van Frankenhuzen 1993, Syngenta 2007, Supplement 20).

Plants that were genetically engineered to express the Cry1Ab protein have a history of safe use in the U.S. Since the mid-1990s, corn and cotton lines have been commercialized without substantiated reports of significant deleterious impacts on non-target organisms (EPA 2008, Mendelson 2003, OECD 2007, USDA-APHIS 2009). The use of transgenic cotton producing the Cry1Ab proteins has been shown to reduce the use broad spectrum insecticides[7] without significant impacts on diversity of non-target insects (Cattaneo 2006, Dively 2005, Marvier 2007, Naranjo 2005, Romeis 2006, Torres 2005 & 2007, Whitehouse 2005). COT67B is expected to be similar with respect to the low potential harm to the environment (Syngenta 2007, Appendix 6, Supplements 12 through 19 of the petition, letter from Syngenta dated July 20, 2007 (which summarizes the data from the referenced Supplements), EPA 2008). Because Cry1Ab receptors are not present in non-target birds and mammals (Hofmann 1988a and 1988b, Shimada 2006a and 2006b, Van Rie 1989 and 1990), these insecticidal proteins are not expected to adversely affect non-target invertebrate and vertebrate organisms (EPA 2008).

Syngenta submitted data from laboratory and field studies on non-target representative species, and other peer reviewed studies that provide evidence for the lack toxicity of Cry1Ab (Syngenta 2007, Appendix 6, Supplements 12 through 19, letter from Syngenta dated July 20, 2007 (which summarizes the data from the referenced Supplements), EPA 2008). Assessment of insecticidal transgenic crops include laboratory tests with indicator test species to determine potential toxicity at doses of the toxin higher than would be anticipated under field conditions (Rose 2007). Selection of representative indicator test species was based upon the potential for exposure to Cry1Ab. Syngenta submitted non-

[7] Broad spectrum insecticides are chemical insecticides which kill insects that are causing injury to plants and also kill other insects that are not causing injury to the plant. Insects that are inadvertently killed by the application of insecticide are called “non-target” insects. Because the Cry1Ab protein is specific for a narrow range of insects, use of Cry1Ab to control plant pests is recognized as being benifical to the survival of non-target insects (EPA 2008).

target data for two above-ground arthropods (insidious flower bug (*Orius insidious*) and spotted ladybird beetle (*Coleomegilla maculate*)), two soil dwelling arthropods (rove beetle (*Aleochara bilineata*) and springtail (*Folsomia candida*)), a pollinator (honeybee (*Apis mellifera*)), a bird (Bobwhite quail (*Colinus virginianus*)), a mammal (mouse (*Mus musculus*)), an aquatic invertebrate (water flea (*Daphnia magna*)), and a fish (catfish (*Ictalurus punctatus*)). The data submitted in the petition indicate that no significant adverse effects were observed at the maximum test dose for any of the tested species. Other research has also shown no direct adverse effects on insectivores (insects that eat insects) in field and laboratory studies with transgenic plants expressing Cry1Ab (Pilcher 1997, Marvier 2007, Romeis 2004, Romeis 2006). Exposure of aquatic organisms is likely very low because cotton pollen is large, sticky, and is not transported long distances by the wind (Thies 1953). Seed and plant debris are not expected to be readily transported via overland runoff or wind to aquatic habitats.

Based on the above information, APHIS believes that a determination of nonregulated status for COT67B will have no adverse effects on non-target organisms.

Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if event COT67B is unlikely to pose a plant pest risk. Based on the information provided by the applicant and the lack of plant pest risk from the inserted genetic material, weedy characteristics, atypical responses to disease or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, and horizontal gene transfer, APHIS has concluded that event COT67B is unlikely to pose a plant pest risk.

References

An YQ, McDowell JM, Huang S, McKinney EC, Chambliss S, Meagher RB (1996) Strong, constitutive expression of the *Arabidopsis* ACT2/ACT8 actin subclass in vegetative tissues. The Plant Journal 10:107-121.

Arizona (2009) Arizona Game & Fish Department Species Lists. http://www.azgfd.gov/w_c/edits/hdms_species_lists.shtml Accessed August 27, 2009.

Benson L, Darrow RA (1981) Trees and Shrubs of the Southwestern Deserts (p 102). The University of Arizona Press, Tucson, AZ.

Brown JR (2003) Ancient horizontal gene transfer. Genetics. 4:121-132.

Carpenter J, Felsot A, Goode T, Hammig M, Onstad D, and Sankula S (2002) Comparative Environmental Impacts of Biotechnology-Derived and Traditional Soybean, Corn, and Cotton Crops. Council for Agricultural Science and Technology, Ames, IA.

Cattaneo MG, Yafuso C, Schmidt C, Huang C-Y, Rahman M, Olson C, Ellers-Kirk C, Orr BJ, Marsh SE, Antilla L, Dutilleul P, Carrière Y (2006) Farm-scale evaluation of the impacts of transgenic. Proceedings of the National Academy of Sciences of the United States of America 10:7571–7576.

Coile NC, Garland MA (2003) Notes on Florida's Endangered and Threatened Plants. Bureau of Entomology, Nematology & Plant Pathology – Botany Section Contribution No. 38, 4th edition.

DeJoode DR, Wendel JF (1992) Genetic diversity and origin of the Hawaiian Islands cotton, *Gossypium tomentosum*. American Journal of Botany 79:1311-1319.

Depicker A, Stachel S, Dhaese P, Zambryski, Goodman HM (1982) Nopaline synthase: transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics 1:561-573.

Dively GP (2005) Impact of transgenic VIP3A x Cry1Ab lepidopteran-resistant field corn on the non-target arthropod community. Environmental Entomology 34:1267-1291.

EPA (2008) Biopesticides Registration Action Document *Bacillus thuringiensis* modified Cry1Ab (SYN-IR67B-1) and Vip3Aa19 (SYN-IR102-7) insecticidal proteins and the genetic material necessary for their production in COT102 XCOT67B cotton.

Florida (2003) Notes on Florida's Endangered and Threatened Species. http://www.fl-dof.com/forest_management/plant_conservation_index.html Accessed August 27, 2009.

Geiser M, Schweitzer S, Grimm C (1986) The hypervariable region in the genes coding for entopathogenic crystal proteins of *Bacillus thuringiensis*: nucleotide sequence of the *durhd1* gene of subsp. *kurstaki* HD1. Gene 48:109-118.

Geiser M, Moser J (1991) Canadian Patent No. 2,035,199. Temperature-stable *Bacillus thuringiensis* toxin.

Glare TR, O'Callaghan M (2000) *Bacillus thuringiensis* Biology, Ecology and Safety. John Wiley & Sons, Chichester. 350pp.

Hawaii (2001) State of Hawaii Threatened and Endangered Plants http://www.state.hi.us/dlnr/dofaw/pubs/TEplant.html Accessed August 27, 2009.

Hofmann C, Luthy P, Hutter R, Pliska V (1988a) Binding of the delta endotoxins from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). European Journal of Biochemistry 173:85-91.

Hofmann C, Vanderbruggen H, Höfte H, Van Rie J, Jansens S, Van Mellaert H (1988b) Specificity of *Bacillus thuringiensis* δ-endotoxins is correlated with the presence of

high-affinity binding sites in the brush border membrane of target insect midguts. Proceedings of the National Academy of Sciences 85:7844-7848.

Holm LG, Plucknett DL, Pancho JV, Herberger JP (1977) The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu. 609 pp.

Holm L, Doll J, Holm J, Pancho J, Herberger J (1997) World Weeds: Natural Histories and Distribution. John Wiley & Sons, New York. 1129 pp.

Hooykaas PJJ, Schilperoort RA (1992) *Agrobacterium* and plant genetic engineering. Plant Molecular Biology 19:15-38.

Kaneko T *et al.* (2000) Complete genome structure of the nitrogen-fixing xymbiotic bacterium *Mesorhizobium loti*. DNA Research. 7:331-338.

Kaneko T *et al.* (2002) Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Research 9:189-197

Kearney T, Peebles RH (1960) Arizona Flora. University of California Press, Berkeley, CA. page 553.

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

Khan AH, Afzal M (1950) Natural crossing in cotton in Western Punjab IV. Agents of natural crossing. Agronomy Journal 42:236-238.

Klee H (2000) A guide to *Agrobacterium* binary Ti vectors. Trends in Plant Science 5:446-451.

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

Koziel, MG, Desai, N., Lewis, KS, Kramer, VC, Warren, GW, Evola, SV, Crossland, LD, Wright, MS, Merlin, EJ, Launis, KL, Rothstein, SJ, Bowman, CG, Dawson, JL, Dunder, EM, Pace, GM, Suttie, J. (1997) United States Patent No. 5,625,136. Synthetic DNA sequence having enhanced insecticidal activity in maize.

Marvier M, McCreedy C, Regetz J, Kareiva P (2007) A Meta-Analysis of Effects of *Bt* cotton and maize on non-target invertebrates. Science 316:1475-1477.

McGregor SE (1976) Insect Pollination of Cultivated Crop Plants. Agriculture Handbook No. 496. U.S. Government Printing Office. Washington, DC.

Mendelsohn M, Kough J, Vaituzia Z & Matthew K (2003) Are *Bt* crops safe? Nature Biotechnology 21:1003-1009.

Meyer VG (1974) Interspecific cotton breeding. Economic Botany 28:56-60.

Muenscher WC (1980) Weeds. Cornell University Press, Ithaca, New York. 586 pp.

Naranjo SE (2005) Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. Environmental Entomology 34:1211-1223.

NAS (2004) National Academy of Sciences. Safety of genetically engineered foods: Approaches to assessing unintended health effects. National Academy Press, Washington, D.C.

NCC (2008) National Cotton Council. Beltwide Cotton conference. January 8-11, 2008 Nashville, TN.

NCBI (2007) Entrez Nucleotide Database, including GenBank.

OECD (2004) Consensus Document of Compositional Considerations for New Varieties of Cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key food and Feed Nutrients and Anti-Nutrients.

OECD (2007) Consensus document of safety information on transgenic plants expressing *Bacillus thuringiensis* – derived insect control proteins.

OECD (2008) Consensus document of the biology of cotton (*Gossypium* spp.).

OMRI (2009) Organic Materials Review Institute Products List http://www.omri.org/OMRI_products_list.html Accessed August 28, 2009.

Pilcher CD, Obrycki JJ, Rice ME, Lewis LC (1997) Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environmental Entomology 26:446-454.

Pleasants, JM, Wendel, JF (2010). Reproductive and pollination biology of the endemic Hawaiian cotton, *Gossypium tomentosum* (Malvaceae). Pacific Science 64(1): 45-55.

Reed CF (1977) Economically important foreign weeds. Agricultural Handbook No. 498, Washington, D.C. 746 pp.

Rose R (ed.) (2007) White paper on tier-based testing for the effects of proteinaceous insecticidal plant-incorporated protectants on non-target arthropods or regulatory risk assessments. Available at: http://www.aphis.usda.gov/brs/pdf/NTO_White_Paper_1.pdf

Romeis J, Dutton A, Bigler F (2004) *Bacillus thuringiensis* toxin (Cry1Ab has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuoptera: Chrysopidae). Journal of Insect Physiology 50:175-183.

Romeis J, Miessle M, Bigler F (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nature Biotechnology 24:63-71.

Romeis J, Shelton AM, Kennedy GG (2008) Integration of Insect-Resistant Genetically Modified Crops with IPM Programs. Springer 441pp.

Shelton AM, Zhao J-Z, Roush RT (2002) Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. Annual Review of Entomology 47:845-881.

Shimada N, Murata H, Mikami O, Yoshioka M, KS Guruge KS, Yamanaka N, Nakajima Y, Miyazaki S (2006a) Effects of feeding calves genetically modified corn Bt11: A clinico-biochemical study. Journal of Veterinary Medical Science 68:1113-1115.

Shimada NK, Miyamoto K, Kanda K, Murata H (2006b) *Bacillus thuringiensis* insecticidal Cry1Ab toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: an in vitro study. In Vitro Cellular & Developmental Biology - Animal 42:45-49.

Syngenta (2007). Petition for the determination of nonregulated status for Event COT67B. Submitted by J. N. Reed, Regulatory Affairs Manager. Syngenta Biotechnology, Inc. (See Table http://www.aphis.usda.gov/biotechnology/not_reg.html).

Thies SA (1953) Agents concerned with natural crossing of cotton in Oklahoma. Agronomy Journal 45:481-484.

Torres JB, Ruberson JR (2005) Canopy- and ground-dwelling predatory arthropods in commercial *Bt* and non-*Bt* cotton fields: patterns and mechanisms. Environmental Entomology 34:1242-1256.

Torres JB, Ruberson JR (2007) Abundance and diversity of ground-dwelling arthropods of pest management importance in commercial *Bt* and non-*Bt* cotton fields. Annals of Applied Biology 150:27-39.

University of Hawaii (2001) http://www2.hawaii.edu/~eherring/hawnprop/gos-tome.htm Accessed August 28, 2009.

USDA-AMS (2009) Cotton Varieties Planted 2009 Crop. Memphis, TN

USDA-APHIS (2009) Petitions of Nonregulated Status http://www.aphis.usda.gov/brs/not_reg.html Accessed August 27, 2009

USDA-APHIS (2008) Pink Bollworm Eradication Plan in the U.S. Grefenstette B, El-Lissy O, Staten RT.

USDA-ERS (2009) Adoption of Genetically Engineered Crops in the U.S.: Upland Cotton Varieties

USDA-ERS (2006) The First Decade of Genetically Engineered Crops in the United States. Economic Information Bulletin Number 11.

USDA-NRCS (2006) Federal Noxious Weeds http://plants.usda.gov/java/noxious?rptType=Federal Accessed August 27, 2009

USDA-NASS (2009) http://www.nass.usda.gov/index.asp Accessed August 28, 2009

USFWS (2009a) Florida Species Accounts http://www.fws.gov/northflorida/ Accessed August 27, 2009

USFWS (2009b) Threatened and Endangered Species http://ecos.fws.gov/tess_public/ Accessed August 28, 2009

Van Frankenhuyzen K (1993) The Challenge of *Bacillus thuringiensis*. In: Entwistle PF (ed.) *Bacillus thuringiensis*: an Environmental Biopesticide: Theory and Practice. Pp 1-35. John Wiley & Sons.

Van Rie J, Jansens S, Höfte H, Degheele D, and Van Mellaert H (1989) Specificity of *Bacillus thuringiensis* δ-endotoxins, importance of specific receptors on the brush border membrane of the mid-gut of target insects. European Journal of Biochemistry 186:239-247.

Van Rie J, Jansens S, Höfte H, Degheele D, and Van Mellaert H (1990) Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta endotoxins. Applied and Environmental Microbiology 56:1378-1385.

Wagner WL, Herbst DR, Sohmer SH (1999) Manual of the Flowering Plants of Hawaii. University of Hawaii Press Bishop Museum.

Whitehouse MEA, Wilson LJ, Fitt GP (2005) A comparison of arthropod communities in transgenic *Bt* and conventional cotton in Australia. Environmental Entomology 34:1224-1241.

Williams MR (2008) Cotton Insect Loss Estimates. 2008 Beltwide Cotton Conferences, Nashville, Tennessee, January 8-11, 2008

Wood DW, *et al.* 2001. The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science. 294:2317-2323.

Wunderlin RP, Hansen BF (2004) Atlas of Florida Vascular Plants. [S. M. Landry and K. N. Campbell (application development), Florida Center for Community Design and Research.] Institute for Systematic Botany, University of South Florida, Tampa. Available at: http://www.plantatlas.usf.edu/