

Plant Pest Risk Assessment for Event 5307 Corn

Syngenta Biotechnology, Inc. has petitioned APHIS (APHIS number 10-336-01p) for a determination that genetically engineered (GE) corn (*Zea mays* L. subsp. *mays*) Event 5307 is unlikely to pose a plant pest risk (Syngenta 2011) 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 2000¹. This plant pest risk assessment was conducted to determine whether corn Event 5307 is unlikely to pose a plant pest risk under full deregulation.

Event 5307 was produced by transformation of an immature corn embryo of line NP222 with *Agrobacterium tumefaciens*. Because *A. tumefaciens* is a plant pest and some of the regulatory sequences (promoter from Cestrum yellow leaf curling virus and terminator from *A. tumefaciens*) used to facilitate expression of the genes in corn were derived from plants pests, this corn has been considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of Event 5307 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 Event 5307 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is not a plant pest risk, then APHIS has no regulatory authority over that organism.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk characteristics. APHIS regulation 7 CFR 340.6(c) specifies the information needed for consideration in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, any impacts on the weediness of any other plant with which it can interbreed, and transfer of genetic information to organisms with which it cannot interbreed.

The Environmental Assessment (EA) for this petition considers whether agricultural or cultivation practices for Event 5307 may result in impacts on the environment. A thorough assessment of the effects of the determination on non-target and beneficial organisms, and threatened and endangered species is included in the EA.

Development of Event 5307 Corn

Corn is the most widely produced feed grain in the U.S., accounting for more than 90

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."

percent of the total value and production of feed grain (USDA-ERS 2011). 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 corn expressing Cry proteins derived from *Bacillus thuringiensis* (*Bt*), commonly referred to as *Bt* corn, have a long history of safe use without adverse human health or environmental effects and provide an option for the control of coleopteran insect pests (NAS 2004). The *Bt* proteins used in this product called eCry3.1Ab controls corn rootworm, which is a highly destructive pest responsible for the single largest use of conventional insecticides in the United States (EPA 2007a).

Corn rootworms have long been the major economic pests of corn in the U.S. (Calvin 2003). Syngenta has genetically engineered corn to express an eCry3.1Ab protein for use in the control of coleopteran pests. Event 5307 deters feeding by several insects known to cause significant damage to corn in the U.S. These include: western corn rootworm (*Diabrotica virgifera virgifera* Le Conte), northern corn rootworm (*D. longicornis barberi* Smith and Lawrence) and Mexican corn rootworm (*D. virgifera zea* Krysan and Smith) (Peairs and Pilcher 2010).

GE corn containing insect-resistant traits, first approved in the U.S. in 1996, has been widely adopted by U.S. growers (Figure1). Adoption of stacked varieties (those containing both herbicide and insect tolerance traits) has accelerated in recent years. Planting of stacked corn made up 49 percent of corn acres in 2011 (Figure 2).

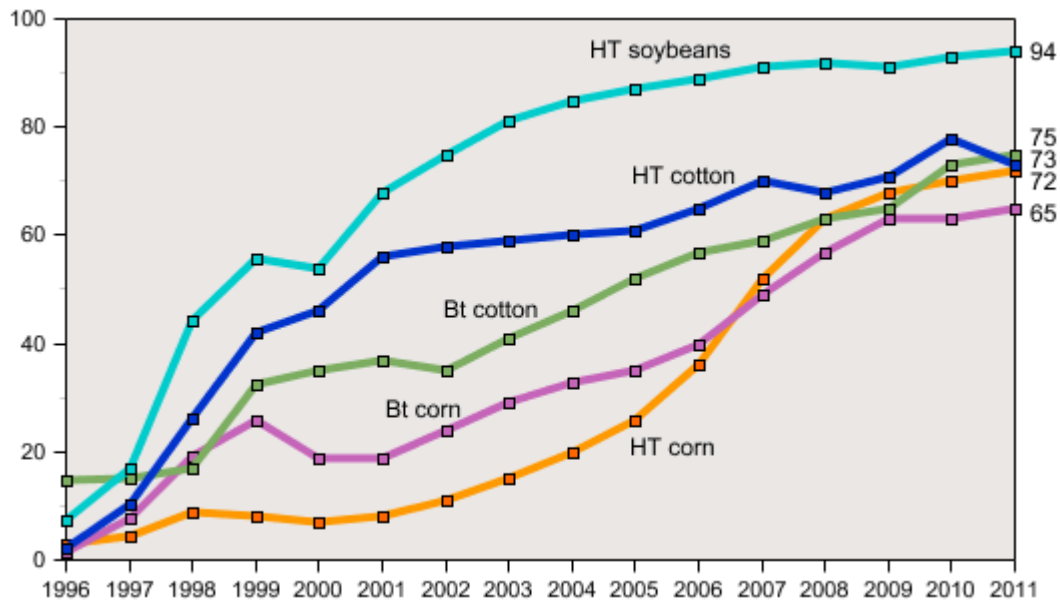


Figure 1. Percent of acreage of genetically engineered crops in the U.S., 1996-2011 (USDA ERS 2011).

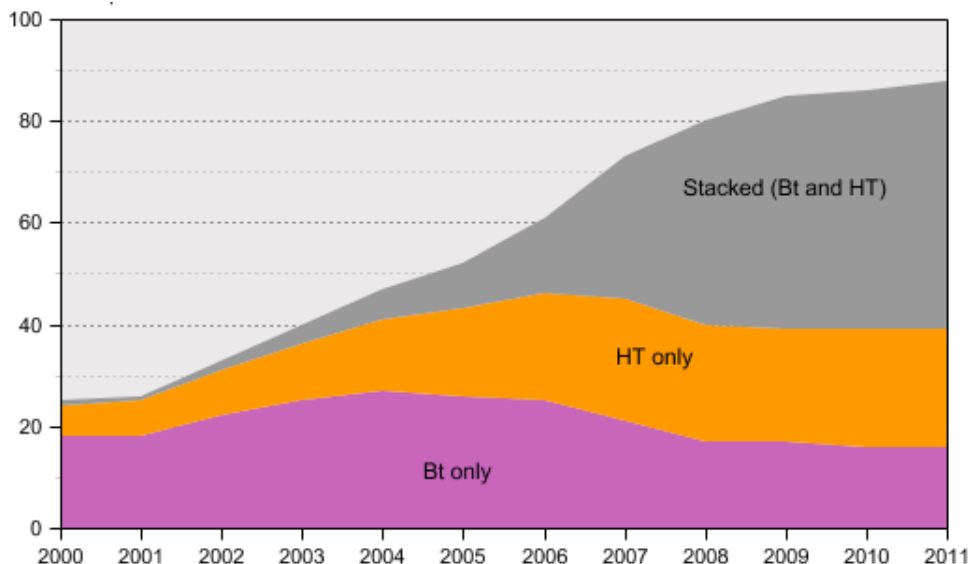


Figure 2. Percent of planted acres of corn in the U.S. by trait, 2000-2011 (USDA-ERS 2011).

The eCry3.1Ab protein is a chimeric *Bacillus thuringiensis* protein, composed of portions of Cry1Ab and mCry3A proteins both of which are EPA-registered Plant Incorporated Protectants (75 FR 34040-34045).

On June 16, 2010 EPA issued a temporary exemption from the requirement of tolerance for eCry3.1Ab protein in corn that expires June 1, 2012 (EPA 2010). Syngenta filed for registration of pesticidal use of the eCry3.1Ab protein in corn on January 21, 2011 (EPA 2011). Phosphomannose isomerase (PMI), the selectable marker protein produced by Event 5307 corn plants, is exempt from food and feed tolerances (EPA 2007b). Syngenta initiated a voluntary pre-market consultation process with FDA and submitted a safety and nutritional assessment for Event 5307 corn in January 2011 (File BNF 000128).

Description of the Modification

Event 5307 corn was produced by transformation using disarmed *A. tumefaciens* (Syngenta 2011, pp. 26 - 28, Figures III-1, III-2). Immature embryos of Syngenta inbred corn line NP2222 (Plant Variety Protection Certificate 200200071; USDA-AMS 2004) were infected with *A. tumefaciens* strain LBA4404 containing plasmid SYN12274. Plants containing the introduced DNA were selected based on survival on a mannose substrate due to the expression of phosphomannose isomerase (Syngenta 2011, p. 27, Figure III-2).

The plasmid SYN12274 contained two gene expression cassettes flanked by the right and left border of T-DNA from the *A. tumefaciens* Ti-plasmid (Syngenta 2011, pp. 28, 35-36, Figure III-1, Table IV-1).

eCry3.1Ab – expression cassette consists of three genetic elements (Syngenta 2011, Figure III-1, Table IV-1).

- The Cestrum Yellow Leaf Curling Virus (CmYLCV, *Caulimoviridae* dsDNA plant virus²) genomic full-length transcript promoter (Hohn 2007; Stavone 2003).
- The *ecry3.1Ab* gene consists of a fusion between the 5' end (Domain I, Domain II and 15 amino acids of Domain III) of a modified *mcry3A* gene from *B. thuringiensis* subsp. *tenebrionis* and the 3' end (Domain III and variable region 6) of a *cry1Ab* (Syngenta 2011, Table IV-1, Figure IV-1; Walters 2010).
- The nopaline synthase (NOS) terminator from *A. tumefaciens* (Depicker 1982).

Phosphomannose isomerase – expression cassette consists of three genetic elements (Syngenta 2011, Figure III-1, Table IV-1).

- The polyubiquitin promoter from *Z. mays* (Christensen 1992).
- The *pmi* gene from *Escherichia coli* strain K-12 (Negrotto 2000).
- The NOS terminator from *A. tumefaciens* (Depicker 1982).

Data from Southern blot analysis was provided and reviewed by APHIS that showed that Event 5307 contains: (1) a single copy of the CmYLCV promoter (Syngenta 2011, Figures V-4, V-5, Table V-2); (2) a single copy of the *ecry3.1Ab* gene (Syngenta 2011, Figures V-2, V-3, Table V-1); (3) two copies of the NOS terminator (Syngenta 2011, Figures V-10, V-11, Table V-5); and (4) a single copy of the *pmi* gene (Syngenta 2011, Figures V-6, V-7, Table V-3). Southern blot analysis demonstrated the absence of the plasmid backbone elements (Syngenta 2011, Figures V-12, V-13, Table V-6). Data from Southern analysis was provided and reviewed by APHIS that demonstrates stable integration and inheritance of the 5307 corn insert over four generation (Syngenta 2011, Figures V-14, V-15, V-16, Table V-7).

Potential of 5307 to Become Invasive and/or a Weed

Corn possesses few of the characteristics of those plants that are notably successful as weeds (Baker 1965; Keeler 1989). In the U.S., corn is not listed as a weed (Crockett 1977; Holm 1991; Muenscher 1980), nor is it present in the Federal noxious weed list (7 CFR part 360³). Furthermore, corn is grown throughout the world without any report that it is a serious weed or that it forms persistent feral populations (Gould 1968). Like many domesticated crops, corn seed from a previous year's crop can overwinter and germinate the following year. For instance, the appearance of corn seedlings in soybean fields following a corn crop is a common occurrence. Manual or chemical measures are often applied to remove these volunteers, but the plants that are not removed do not typically result in feral populations in following years.

² <http://ictvdb.cumc.columbia.edu/servlet/Virus?id=944>

³ http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf

APHIS assessed whether Event 5307 is any more likely to become a weed than a near-isogenic non-transgenic corn line. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of the unique characteristics of Event 5307 (Syngenta 2011 Table VII-1, Appendix A Table A-1). Syngenta has been conducting agronomic field trials of Event 5307 since 2005 at locations that provide a range of environmental conditions representative of where Event 5307 is expected to be grown (Syngenta 2011, Appendix A, Table A-1). Field trial data indicated that Event 5307 does not exhibit characteristics that would cause it to be weedier than the parental corn line (Syngenta 2011 Tables VII-5, VII-6, VII-7, VII-8, VII-9, VII-10). No differences in phenotypic characteristics that might contribute to enhanced weediness were observed between Event 5307 and control lines for the wide range of phenotypic endpoints assessed in these field trials or in greenhouse or laboratory experiments (Syngenta 2011, Tables VII-7, VII-8, VII-9, VII-10, VI-12, Figure VII-1). There was no increase in weediness potential as measured by differences in seed germination, dormancy, plant emergence, plant height, ear height, stalk lodging, barren plants, timing of pollen shed or silking, pollen viability and morphology, and yield (Syngenta 2011, Tables VII-7, VII-8, VII-9, VII-10, VI-12, Figure VII-1). None of the measure attributes showed any differences relative to its comparator, suggesting that Event 5307 is not weedier than current corn cultivars.

These results on growth characteristics, seed production and germination indicate that Event 5307 is not significantly different from its comparators. There is no indication that Event 5307 possesses a selective advantage that would result in increased weediness. As with other corn varieties, Event 5307 lacks the ability to persist as a troublesome weed, and there would be no significant impact on current weed management practices for corn cultivation.

Potential for Gene Flow and Gene Introgression from Event 5307 into Sexually-Compatible Relatives

Gene flow from crops to wild relatives may have the potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower, and a few other crops (Ellstrand 1999). 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 (Soltis and Soltis 1993; Rieseberg 1997) and even in existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg 1997). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars.

APHIS evaluated the potential for gene introgression to occur from Event 5307 to sexually compatible wild relatives and considered whether such introgression would result in increased weediness (Syngenta 2011, Figure III-3, Table III-1). Cultivated corn is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum* (OECD 2003). The closest wild relatives of corn, various *Zea* species referred to as teosinte, are normally confined to the tropical and

subtropical regions of Mexico, Guatemala, and Nicaragua. In the U.S. a fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida (USDA PLANTS Database⁴, accessed 8/23/2011).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America. Three of these species (*T. dactyloides*, *T. floridatum*, and *T. lanceolatum*) exist as wild and/or cultivated species in the continental U.S.; and two taxa (*T. faciculatum* and *T. latifolium*) also occur in Puerto Rico (USDA PLANTS Database, accessed 8/23/2011). Though many of these species occur where corn might be grown, gene introgression from Event 5307 under natural conditions is highly unlikely. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility and none of them typically withstand even the mildest winters (Galinat 1988; Ellstrand 2007). First-generation hybrids are generally less fit for survival and dissemination in the wild and show substantially reduced reproductive capacity, which thus acts as a significant constraint to introgression. Therefore, USDA has concluded that adverse consequences of gene flow from Event 5307 to wild or weedy species in the U.S. are highly unlikely.

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

Based on the data provided by the applicant and existing literature, APHIS evaluated the potential for plant pest-related impacts from Event 5307 on non-target or beneficial organisms. The genes introduced into Event 5307 result in the production of phosphomannose isomerase (PMI) and eCry3.1Ab proteins.

The *pmi* gene, which was introduced along with the *ecry3.1Ab* gene via the same pSYN12274 transformation vector, was employed as a selectable marker during the process of regenerating plant material following transformation (Syngenta 2011, p. 40, Figure V-1). The *pmi* gene was isolated from *E. coli*, which is a common intestinal bacterium and is considered a non-allergenic source of proteins (FAO/WHO 2001). The PMI protein is widely distributed in nature and has been found to be expressed in enteric bacteria, fungi, insects, some species of plants, and mammals (EPA 2004, Slein 1950). Additionally, EPA has granted an exemption from the requirement of a tolerance for the PMI protein as an inert ingredient in all plants (EPA 2004).

The *ecry3.1Ab* gene from *Bacillus thuringiensis* expressed in Event 5307 is a chimeric gene composed of portions of the N-terminus of *mcry3A* gene and the C-terminus of *cry1Ab* gene (Syngenta 2010, Figures IV-1A, IV-1B). For decades, microbial products containing *B. thuringiensis* have been used to control insect pests on a commercial scale and for home garden applications (Glare and O'Callaghan 2000; OMRI 2011; Shelton 2002). Because Cry protein receptors are not present in non-target organisms (Glare and O'Callaghan 2000; Hofmann 1988a; Hofmann 1988b; Shimada 2006a; Shimada 2006b; Van Rie 1989; Van Rie 1990), these insecticidal proteins are not expected to adversely

⁴ http://plants.usda.gov/java/county?state_name=Florida&statefips=12&symbol=ZEME

affect non-target invertebrate and vertebrate organisms (Glare and O'Callaghan, 2000; EPA 2007a; EPA 2008).

Since the commercialization of *Bt* crops, a large number of field studies published in the scientific literature have addressed the issue of long range effects of cultivation of Cry proteins on the invertebrate community structure where *Bt* crops are grown. The use of transgenic crops producing Cry proteins has been shown to reduce the use of broad spectrum insecticides⁵ without significant impacts on non-target organisms (Cattaneo 2006; Dively 2005; Marvier, 2007; Mendelsohn 2003; Naranjo, 2005; OECD 2007; Romeis 2006; Torres and Ruberson, 2005; Torres and Ruberson, 2007; Yu 2011, Whitehouse 2005). Specifically, a meta-analysis⁶ of the data collected from 42 field studies indicated that non-target invertebrates are generally more abundant in *Bt* cotton and *Bt* corn fields than in non-transgenic fields managed with insecticides (Marvier 2007). In addition, a comprehensive review of short and long term field studies on the effects of invertebrate populations in *Bt* corn and cotton fields indicated that no unreasonable adverse effects are taking place as a result of wide scale *Bt* crop cultivation (Sanvido 2007). Environmental exposure of non-target aquatic organisms is generally dependent upon the input of crop debris into streams and waterways. Because of the short-term exposure and rapid degradation of Cry proteins, there is limited opportunity for exposure 2 weeks after a Cry protein is introduced into the environment by crop debris or pollen shed (Jensen 2010; Wolt 2010). Therefore, the level of Cry proteins found in any surrounding aquatic ecosystem is expected to be below that at which biological activity would be observed (Jensen 2010, Syngenta 2010).

Assessment of insecticidal transgenic crops should include laboratory tests with indicator species to determine potential toxicity at doses of the toxin higher than would be anticipated under field conditions (Rose 2007). Syngenta submitted data from laboratory and field studies on non-target representative species at exposure levels in excess of that anticipated in the environment (Syngenta 2010, pp. 134-152). Data was submitted for two above-ground arthropods (insidious flower bug (*Orius insidiosus*)) and spotted ladybird beetle (*Coleomegilla maculata*)), two soil dwelling arthropods (rove beetle (*Aleochara bilineata*) and ground or carabid beetle (*Poecilus cupreus*)), a soil dwelling annelid (earthworm (*Eisenia fetida*)), a pollinator (honeybee (*Apis mellifera*)), two birds (Bobwhite quail (*Colinus virginianus*) and chicken (*Gallus gallus domesticus*)), a mammal (mouse (*Mus musculus*)), an aquatic arthropod (fresh water shrimp (*Gammarus fasciatus*)), 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.

⁵ 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.

⁶ Meta-analysis combines the results of several studies that address a set of related research hypotheses. The general aim of a meta-analysis is to more powerfully estimate the true "effect size" as opposed to a smaller "effect size" derived in a single study under a given single set of assumptions and conditions.

Event 5307 is expected to be similar with respect to the low potential harm to the environment (Syngenta 2010). Based on the above information, APHIS has concluded that adverse impacts to non-target organisms exposed to Event 5307 corn are unlikely.

Potential for Transfer of Genetic Information with which Event 5307 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, FDA has evaluated horizontal gene transfer from the use of antibiotic resistance genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA 1998). Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no plant pest risk.

Potential for Event 5307 to have Altered Disease and Pest Susceptibilities

APHIS assessed whether Event 5307 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 Event 5307 field trials.

Syngenta collected field trial data on Event 5307 from 2005 through 2009 (Syngenta 2011, Appendix A). Except for differences in rootworm damage, no observations of differences were observed in pest damage or in disease susceptibility (Syngenta 2011, Table III-1, Figures III-3, VIII-1, VIII-2, p. 101). In field trials conducted in 2007 where gray leaf spot and northern corn leaf blight was present, Event 5307 did not show increased susceptibility to gray leaf spot or northern corn leaf blight diseases (Syngenta 2011, Table VII-8). In 2009, no significant differences were observed in the disease ratings for eyespot, southern corn leaf blight and gray leaf spot (Syngenta 2011, Table VII-11).

The *Agrobacterium* transformed plants used in the generation of Event 5307 were treated with an antibiotic to kill the *Agrobacterium* cells (Syngenta 2011, p. 26). Furthermore, DNA sequences derived from plant pests that were incorporated in Event 5307 do not result in the production of infectious agents or disease symptoms in plants, and so it is unlikely that Event 5307 could pose a plant pest risk. The description of the genetic modifications, including genetic elements, expression of the gene product and their functions for Event 5307 has been summarized above.

APHIS considered whether corn constituents that differed between Event 5307 corn and other corn varieties might be sufficient to lead to increased susceptibility to pathogens or

pests. The data presented in the petition indicate no difference in compositional and nutritional quality of Event 5307 compared to conventional corn, apart from the presence of the eCry3.1Ab and PMI proteins (Syngenta 2011). None of the values for seed and forage composition characteristics were outside the range of natural variability of conventional corn seed and forage (Syngenta 2011, Tables VII-13, VII-14, VII-15, VII-16, VII-17, VII-18, VII-19, VII-20, VII-21). Therefore, the composition of Event 5307 is not biologically different than conventional corn (with the exception of the eCry3.1Ab and PMI proteins). Based on the known functions and mechanism of actions of these proteins (summarized in Syngenta 2011), none of these proteins are expected to directly alter susceptibility to plant pests. Thus, Event 5307 is expected to be susceptible to the same pests as conventional corn except for a reduction in rootworm damage.

Corn is not a plant pest in the U.S., and the introduced DNA in Event 5307 is unlikely to pose a plant pest risk. Based on the analysis of genetic modifications and their functions and field testing data submitted by the petitioner, APHIS concludes that there are no significant differences between Event 5307 corn and the non-transgenic counterparts relative to pest and disease susceptibility.

Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if Event 5307 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 5307 is unlikely to pose a plant pest risk.

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