Plant Pest Risk Assessment for HCEM485 Corn

APHIS' authority to regulate genetically engineered (GE) organisms under the Plant Protection Act (PPA) (7 U.S.C. Sec 7701 *et seq.*) is limited to those GE organisms that are plant pests as defined under Section 403(14) of the PPA (7 U.SC. 7702(14)[1]). APHIS regulations at 7 CFR part 340, which were promulgated in 1987 under the authority of the Federal Plant Pest Act and Plant Quarantine Act, provided APHIS with the mechanism to regulate articles altered or produced through genetic engineering as plant pests and that the Administer considers a plant pest based upon the definition of a regulated article (7 CFR part 340.1). In 1993, 7 CFR part 340 was revised to include a process whereby a person may petition the agency to make a determination that a regulated article should not be regulated (7 CFR part 340.6). Required data and information (as per 7 CFR part 340.6(c)) is submitted to the agency to demonstrate that the article that is the subject of the petition does not pose a greater plant pest risk than the organism from which it was derived and that it should no longer be subject to the regulations of 7 CFR part 340. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, APHIS then has no regulatory authority under 7 CFR Part 340 or the plant pest provisions of the Plant Protection Act to continue to regulate that organism. Additionally, under 7 CFR part 340.6(e), a person may request that APHIS extend a determination of nonregulated status, based on similarity of a regulated article to an antecedent organism which has previously been determined to be unlikely to pose a plant pest risk and is no longer subject to the plant pest provisions of the Plant Protection Act and 7 CFR part 340.

Stine Seed Farm, Inc. (hereafter referred to as Stine Seed), has submitted to APHIS an extension request for a determination of nonregulated status of GE corn (*Zea mays*) event HCEM485, based on similarity to the corn event GA21 for which a petition (97-099-01p) for nonregulated status was approved in 1997 (62 FR 64350). The Stine Seed extension request for HCEM485 corn was originally received on March 4, 2009. A revised version (09-063-01p_a3) received April 1, 2011 (Stine, 2011) was deemed technically complete and is the version of the petition referenced in this document. Both HCEM485 corn and GA21 corn are similarly genetically engineered for resistance to the herbicide glyphosate. This plant pest risk assessment was conducted to determine whether, as with the antecedent GA21 corn, event HCEM485 corn is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. If the submitted extension request provides evidence of sufficient similarity to the antecedent GA21, allowing APHIS to make a determination that HCEM485 is unlikely to pose a

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[1] The term "plant pest" is defined in the PPA as 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.

plant pest risk, HCEM485 and its progeny will no longer be considered regulated articles under regulations at 7 CFR part 340.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of HCEM485 corn and its progeny in the absence of confinement as otherwise afforded under the conditions of 7 CFR part 340 for the introduction of genetically engineered organisms. APHIS examined information submitted by the petitioner (Stine, 2011) as well as information contained in our determination of nonregulated status and the supporting documents for the antecedent organism GA21 (as per 7 CFR part 340.6(c)(4)) related to plant pest risk characteristics, including disease and pest susceptibilities, expression of the gene product, new enzymes or changes to plant metabolism, changes to agricultural practices that might impact plant pest control measures, weediness (including control options) of the regulated article and any impacts on the weediness of other plants, the possibility of effects of the regulated article on non-target beneficial organisms, and potential for plant pest effects from horizontal gene transfer. APHIS also examined information submitted by the petitioner (as per 7 CFR part 340.6(e)(2)) related to the similarity of the regulated article to the antecedent organism GA21. Other issues related to agricultural or cultivation practices (including but not limited to those related to changes in herbicides used to control weeds in corn) and other impacts to the environment are examined in the separately drafted Environmental Assessment for HCEM485 corn.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, June 26, 1986). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with the APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies. The EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. The EPA also sets tolerance limits for residues of pesticides, including herbicides, 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). HCEM485, as well as the antecedent organism GA21, are not engineered to express pesticidal substances, however tolerance levels for residues of glyphosate have already been established for GA21 corn. Supplemental labels are required to allow over-the-top applications of glyphosate herbicide on crops genetically modified for glyphosate resistance and have already been approved for GA21 corn. The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary consultation process (57 FR 22984). Stine Seed has submitted a food and feed safety and nutritional assessment for events HCEM485 on Dec. 27, 2010, and it has been designated BNF 106, and it is currently under review (Ditto, 2012), however a consultation process has been completed for GA21 (BFN 51) and is available for public review via the FDA Completed Consultations on Bioengineered Foods page at www.fda.gov/bioconinventory.

History of Development of HCEM485 Glyphosate Herbicide-Resistant Corn

HCEM485 corn is a GE corn line that has been provided resistance to treatment with the herbicide glyphosate. The particular genetic element conferring this trait is an altered sequence of DNA derived from the genome of corn. Specifically, Stine Seed removed a 6 kilobase (six thousand nucleotide) section of DNA from an inbred corn line, modified the 5-enolpyruvylshikimate-3-phosphate synthase (*epsps*) gene contained within that DNA section, and then reintroduced the DNA into corn. The modified genetic sequence of the *epsps* gene encodes a double mutated 2mEPSPS enzyme product identical to that produced in the non-regulated GE corn line, GA21, that is also resistant to glyphosate (Funke et al. 2009), although the regulatory sequences controlling expression of the gene do differ between HCEM485 and GA21 (see "Description of the Inserted Genetic Material" below).

A petition request for a determination of nonregulated status of the first corn line containing a glyphosate resistance trait produced through the use of biotechnology was approved by APHIS in 1995, and since that time several other corn lines containing glyphosate resistance have also been determined by APHIS to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. In 2011, approximately 72% of corn planted in the United States possessed resistance to an herbicide that was conferred through biotechnology (USDA-ERS, 2011). The primary herbicide resistance trait in use is glyphosate resistance, and the adoption of this trait in other major crops, such as cotton and soybean, is even higher. Weed resistance to herbicides is a concern in agricultural production, and the wide-spread adoption of herbicide resistant crops, especially GE-derived glyphosate-resistant crops, has dramatically changed the approach that farmers take to avoid yield losses from weeds (Vencill et al., 2012).

Glyphosate-resistant crops have become widely adopted since their introduction in the mid-late 1990s for several reasons. Glyphosate works non-selectively on a wide range of plant species, is a relatively low-cost herbicide, facilitates 'no-till' farming practices, and has minimal animal toxicological and environmental impact (Gianessi, 2008; Duke and Powles, 2009)

Stine Seed conducted confined field tests of HCEM485 corn in the United States beginning in 2005 under requested notifications acknowledged by APHIS (Stine 2011, page 50 and Appendix 2), and has provided data in the petition from several of those confined field trials.

If a determination of nonregulated status is extended to HCEM485, this cultivar is expected to add to the options that growers have when deciding on a glyphosate-resistant corn line. It is not expected that the availability of HCEM485 will significantly impact the adoption rate of glyphosate resistant corn crops nor will it greatly impact current or future trends of glyphosate resistance among weed species. These specific issues are discussed in greater detail in the Environmental Assessment that is written for HCEM485.

Description of Inserted Genetic Material in HCEM485 and the Antecedent

The similarity of the transformation process and inserted genetic elements and gene products expressed in HCEM485 corn compared to the antecedent are summarized here and inform the plant pest risk assessment.

HCEM485 corn was produced using an aerosol beam injector (Held, 2004) to introduce a 6.0 kilobase corn genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) corn DNA library. The method of direct DNA introduction is a similar method to the particle bombardment, or "biolistics", approach used to generate the antecedent event GA21. The original DNA from the BAC library was modified to incorporate the desired change to EPSPS functionality, however all genetic material inserted by aerosol beam injection was otherwise entirely derived from corn DNA. All promoter, intron, and terminator sequences that inserted into HCEM485 are the same as those already associated with the native EPSPS-encoding gene in corn. No DNA from plant pests, nor any transformation method or vector involving a plant pest, was used to produce HCEM485 corn. The only modification to the corn DNA sequence is the introduction of two single-nucleotide alterations, which results in two changes to the amino acids (Threonine-102→Isoleucine and Proline-106→Serine) of the expressed EPSPS protein. Corn plants containing the inserted genetic material were initially isolated by growth on selective media containing glyphosate. Stine Seed used a Southern blot analysis to confirm insertion and to estimate the number of copies inserted into the recipient corn product, HCEM485 (Stine 2011, pages 14-18). Evidence from the submitted petition establishes that multiple copies of the modified *epsps* gene were inserted at a single locus (also similar to the antecedent) and that there does not appear to be any truncated or abnormally sized protein products resulting from the inserted genetic material (as determined by western blot analysis), (Stine 2011, pages 20-22).

The notable difference between HCEM485 and GA21 is the inserted non-coding DNA regulatory sequences before and after the sequences encoding the modified EPSPS protein. Whereas all regulatory sequences up- and down-stream of the modified *epsps* in HCEM485 are derived directly from native corn DNA, in GA21 those sequences are also derived from various DNA isolated from other organisms, including rice (*Oryza sativa*), sunflower (*Helianthus annus*) and the bacterium *Agrobacterium tumefaciens* that is listed as a plant pest in 7 CFR part 340.2 (see Stine 2011, Table 1). As APHIS has already approved the petition request for a determination of nonregulated status of GA21 (62 FR 64350, APHIS number 97-099-01p) and therefore GA21 is not considered to present a plant pest risk, these differences in the introduced DNA do not provide any increase in the plant pest risk potential for HCEM485 compared to GA21.

Expression of the Gene Product, Enzymes, or Changes to Plant Metabolism

APHIS assessed whether changes in plant metabolism or composition in HCEM485 are likely to alter plant pest risk. The assessment encompasses a consideration of the expressed modified epsps gene and its effect on plant metabolism, and an evaluation of whether HCEM485 is nutritionally equivalent to other corn, as has been determined for GA21. The modified *epsps* gene inserted does not replace the native, unmodified *epsps* gene or EPSPS protein; both modified and unmodified versions of the gene and protein are present within HCEM485. The introduced gene responsible for conferring the herbicide resistance encodes a form of EPSPS that is insensitive to the herbicide glyphosate, similar to the antecedent GA21. This gene encodes a protein that differs from the native corn EPSPS protein by two key amino acids, which is the same modification as introduced into GA21 (Funke, 2009) making the EPSPS enzyme significantly less sensitive to the presence of glyphosate compared to the native form (see Stine 2011, Section IV for further details of the inserted genetic material and resulting protein product). The engineered change does not impact the essential cellular functions of the EPSPS enzyme, and there is no evidence that the protein contributes to any changes to the agronomic properties, disease susceptibility, or composition of HCEM485 corn compared to other commercially available corn (see "Potential Impacts of Genetic Modifications on Altered Disease and Pest Susceptibilities" below). The only expected difference is the desired change in sensitivity to the herbicide glyphosate. GA21 and its progeny, containing a similar form of modified EPSPS, have been available as nonregulated corn plant varieties since 1997, alongside other varieties of glyphosate resistant corn that also express glyphosate resistant versions of the EPSPS enzyme (e.g. NK603, MON 88017, and MON 802). The wide and successful adoption of these very similar corn varieties establishes that extending a determination of nonregulated status to glyphosate resistant corn line HCEM485 is unlikely to present new plant pest risk issues.

The data presented in the submitted request also indicate no substantive difference in compositional and nutritional or anti-nutritional quality of HCEM485 corn treated with glyphosate at the normal commercial application rate compared to other commercially available corn (Stine 2011, Tables 12 -20). None of the values for the forage and grain composition characteristics were outside the range of natural variability of conventional corn reported by the International Life Sciences Institute Crop Composition Database (ILSI, 2006) and/or in the OECD consensus document on corn composition (OECD, 2002). Stine Seed also evaluated levels of phytosterols, a common component of plants, and did not find significant differences in mean levels of total phytosterols or individual phytosterols (cholesterol, campesterol, stigmasterol, β-sitosterol, stigmastanol) between grain samples from HCEM485 and the combined samples of the control hybrids. Although host plant quality and composition is known to affect herbivore performance and fecundity (Awmack and Leather, 2002) and secondary metabolites in plants can affect defense against microbes (Dixon, 2001), the similar compositional profiles between HCEM485 and other commercial corn cultivars would suggest that pest populations or infestations would not be expected to be altered if a determination of nonregulated status is extended to HCEM485. The collective data and the similarity of HCEM485 to other commercially produced corn including GA21, support the petitioner's claim that HCEM485 corn does not have increased susceptibility to any insects, plant pathogens, or other plant pests.

Potential Impacts of Genetic Modifications on Altered Disease and Pest Susceptibilities

USDA-APHIS assessed whether HCEM485 corn is likely to have significantly increased disease and pest characteristics or susceptibility compared to both nonregulated GE corn line GA21 and non-GE control corn.

Corn is not a plant pest in the United States (USDA-APHIS, 2000) . Furthermore, none of the inserted genetic sequences are derived from any plant pests listed in 7 CFR part 340.2 or pests listed elsewhere in 7 CFR parts 300-399. The description of the genetic modification, including the introduced genetic elements and the resulting expression and function of the modified EPSPS enzyme in HCEM485 corn, has been summarized above. Stine Seed conducted agronomic field trials during the 2007 growing season across 15 locations in 9 states, representative of the major corn-growing areas of the U.S., to evaluate hybrids of HCEM485 in comparison to three control hybrids derived from the same inbred lines used to generate the HCEM485 hybrids. (Stine 2011, pages 23-43). Stine Seed routinely monitored their corn field trials for corn diseases such as Southern rust disease, gray leaf spot, Northern maize leaf blight, common rust and smut (Stine 2011, page 30). There were no meaningful differences in agronomic characteristics that might be indicative of altered sensitivity to pests and diseases between HCEM485 glyphosate resistant corn derived hybrids, and the control counterparts lacking the glyphosate resistant trait under standard corn management practices and without glyphosate treatment. Comparison of disease ratings in one or more of four sites (one each in Iowa, Nebraska, Minnesota, and Illinois) where the individual disease incidence was sufficiently high to warrant assessment did not show any remarkable differences between the HCEM485 and control hybrids. The data submitted by Stine Seed confirms that the inserted genetic material did not confer any altered disease or pest susceptibility to HCEM485 corn.

In summary, all evidence reviewed by APHIS indicate that as with GA21 corn, HCEM485 corn is no more susceptible to plant pathogens and other plant pest species than other commercially available corn, and that there is no added plant pest risk that results from the insertion and expression of the genetic material described in the Petition. It therefore follows that relative to other commercially available corn, including GA21, there should be no indirect plant pest effects on other agricultural products.

Potential Impacts of Associated Changes in Agricultural Practices on Plant Pests

APHIS evaluated whether the genetic modification causes changes in agricultural practices that affect the incidence, severity or control of plant pests. Considering that glyphosate resistant corn already has already achieved a wide-adoption rate among U.S. growers, (approximately 66% of corn acres were treated with glyphosate in 2010, per http://quickstats.nass.usda.gov/, accessed June 2011) and that HCEM485 will be grown in a similar manner to other glyphosate resistant corn, with the same glyphosate application rates, it can be concluded that there will not be any significant changes in agricultural practices if a determination of nonregulated status is extended to HCEM485. While growers may opt for different corn varieties to meet their anticipated needs, the availability of HCEM485 will not alter current or future methods that growers use to produce corn nor any of the methods used to minimize the damage caused by any plant pests. The methods applicable to production of GA21 corn are expected to be applicable to HCEM485 corn.

Potential Impacts Based on the Relative Weediness of HCEM485 Corn

Corn is not typically considered as a weed (Crockett, 1977; Holm et al., 1979; Muenscher, 1980) and is not listed on the Federal noxious weed list (7 CFR part 360). Corn possesses few of the characteristics of notably successful weeds and is grown as a crop throughout the world without any report that it is a serious weed or that it forms persistent feral populations (Baker, 1965; Keeler, 1989). However, corn seed can germinate in undesired locations and would then be considered a weed. A common example of this is the appearance of corn seedlings in soybean fields following a corn crop, associated with reduced soybean yields (Beckett and Stoller, 1988). Compared to other corn varieties, HCEM485 has improved fitness (reduced injury) in the presence of glyphosate herbicides, which translates into fewer options for the removal of volunteer plants. Nonetheless, similar to the antecedent GA21 and other glyphosate tolerant corn varieties, there are many available options for the control of HCEM485 plants so that growers can avoid losses in fields where unwanted HCEM485 might be growing.

APHIS assessed whether HCEM485 corn is any more likely to become a weed than any other corn varieties currently under cultivation. This assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of the unique characteristics of HCEM485 corn evaluated under field conditions. Stine Seed conducted agronomic field trials during the 2007 growing season across 15 locations representative of the major corn-growing areas of the U.S. (Stine 2011, pages 25-30 and Annex 1). Though not all traits were assessed at all locations, up to 17 different agronomic characteristics were assessed, including the percent of barren plants, percent dropped ears, early growth rating, early stand count, seedling vigor, ear height, early root lodging, grain moisture percent, final stand count, heat units to 50% pollen shed, heat units to 50% silking, leaf color ratings, plant height, root lodging rating, stalk lodging rating, grain weight, and grain yield. No differences in phenotypic characteristics that would contribute to enhanced weediness were observed between HCEM485 corn and control lines for the range of phenotypic endpoints assessed. There were some statistically significant differences between hybrids derived from HCEM485 corn and control counterparts. The significant differences that were seen in ear height, plant height, heat units to 50% silking, heat units to 50% pollen shed, grain yield, and final stand count are actually representative of very small differences, compared to the overall range of corn, and these differences did not appear both genotype specific and consistent across tested field locations (Stine 2011, pages 28-30 and Annex 1).

Based on the agronomic field data and literature survey about corn weediness potential, HCEM485 corn lacks the ability to persist as a troublesome or invasive weed. Still, because HCEM485 corn might grow in areas where it is undesired, the GE corn, similar to other corn varieties, would be considered a weed (Beckett and Stoller, 1988). Because the genetic elements inserted into HCEM485 corn confers greater resistance to glyphosate herbicides the measures that can be used to remove volunteer HCEM485 corn are more limited compared to other glyphosate-sensitive corn varieties. Nonetheless, as discussed in the next paragraph, most of the currently used control measures for removing volunteer corn are also available for the removal of HCEM485 corn. Furthermore, similar glyphosate resistant corn plants, such as GA21 and its progeny, are already widely distributed for agricultural production and growers are familiar with procedures for removing any unwanted corn that is glyphosate resistant.

Corn is commonly grown in rotation with other crop varieties including, but not necessarily limited to, oats, forage crops, peanut, wheat, rye, cotton, and soybean. Corn seed left in a field after harvest is common, and that seed can germinate, or “volunteer,” during the growth of the following rotational crop. If this corn is not destroyed or removed, the subsequent crop planting may have lower yields and reduced value due to competition and mixing with the volunteer corn (Beckett and Stoller, 1988; Beckie and Owen, 2007). The advent and wide adoption of herbicide resistant crops has already changed the approaches growers can adopt to reduce crop yield losses resulting from volunteer corn. For example, glyphosate-resistant soybean plants, grown after glyphosate-resistant corn, can have reductions in soybean yield, due to competition with volunteer corn, if glyphosate is the only weed control method used (Soltani et al., 2006). Similar studies have also been done for corn-cotton rotations (Thomas et al., 2007; Clewis et al., 2008) and corn-wheat rotations (Martin, 2008). Similar to other corn varieties, including the GA21 antecedent, HCEM485 plants or seed left in a field after harvest may be considered a weed when present during the subsequent planting of other crops, including rotational crops such as soybean. When this occurs, farmers have the option to use other herbicides to control the volunteer plants (Deen et al., 2006; Soltani et al., 2006; Thomas et al., 2007; WSSA, 2007; Clewis et al., 2008). Use of any herbicide for the control of volunteer HCEM485 corn is subject to any labeling restrictions established by the EPA, as per FIFRA. Additionally, there are also many non-chemical methods of weed control (tilling, mechanical removal, etc.) that are effective for removing any unwanted HCEM485 corn.

In summary, APHIS found that HCEM485 is no more likely than the antecedent GA21 corn to become a troublesome weed or present weed management issues.

Potential Impacts from Outcrossing (Gene Flow) to Sexually-compatible Wild Relatives

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; Soltis and Soltis, 1993; Rieseberg, 1997; Hegde et al., 2006) . Even in existing floras, the occurrence of hybridization or introgression is reported to be widespread (Knobloch, 1972; 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. 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 other crops (Ellstrand et al., 1999).

Corn is a monoecious species with separate male and female inflorescences that enable cross pollination. Corn is predominantly a wind pollinated outcrosser with occasional bee visitation for pollen. Bees rarely visit female inflorescences (silk). Researchers recognize that: (i) the percent gene flow will vary by population, hybrid or inbred, (ii) the level of gene flow decreases with greater distance between the source and recipient plants; (iii) environmental factors affect the level of gene flow, (iv) corn pollen is viable for a relatively short period of time under field conditions, (v) corn produces ample pollen over an extended period of time, and (vi) corn is not pollinated by insects (pollinating insects, especially bees, are occasional visitors to the tassels but rarely visit silks of corn) (Luna, 2001; Jemison and Vayda, 2002).

APHIS evaluated the potential for gene introgression to occur from HCEM485 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn, or maize, *Zea mays* L. subsp. *mays*, is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum* (OECD, 2003). Wild diploid and tetraploid members of *Zea*, collectively referred to as teosinte, are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua (Wilkes, 1967; Fukunaga, 2005). Teosinte does not appear to be present in the U.S. other than as an occasional botanical garden or research specimen. Although the Plants Profile for *Z. mexicana* (http://plants.usda.gov/java/profile?symbol=ZEME) in the PLANTS Database (USDA-NRCS, 2012) shows distributions in South Carolina, Alabama, Maryland, and Florida, the cited species account (Wunderlin et al., 1996; Wunderlin and Hansen, 2002) as updated (Wunderlin and Hansen, 2008b) only shows distribution of native or naturalized populations in Florida (Miami-Dade, Orange and Levy Counties). The citation for this distribution, however, links to a University of Florida Herbarium Collections Catalog accession number 191119 that lists the specimen as cultivated at a USDA Soil Conservation Service Plant Materials Center in Heranando County and collected in October 21, 1993. Contrary to the Plants Profile for *Z. mexicana*, it is not listed in the citation provided for distribution in Maryland (Brown and Brown, 1984) nor are any *Zea* species or subspecies other than *Z. mays* (corn) listed in Alabama (Kral et al., 2012) . The species account in the PLANTS Database (USDA-NRCS, 2012) written by Hugh Iltis for *Zea perennis* (see http://herbarium.usu.edu/webmanual/info2.asp?name=Zea_perennis&type=treatment, accessed 6/4/2012) describes it as crossing infrequently with *Z. mays* subsp. *mays*, but the hybrids, being triploid, are sterile. While it is described as having been cultivated at research stations in the United States for many years, and Hitchcock (Hitchcock, 1951) reported that it was established at James Island, South Carolina, it is not known if the population has persisted. There are no *Zea* species other than *Zea mays* (corn) found in the comprehensive online South Carolina Plant Atlas hosted by the University of South Carolina A.C. Moore Herbarium and the South Carolina Heritage Trust (South Carolina Heritage Trust, 2011)(available at http://herbarium.biol.sc.edu/scplantatlas.html, accessed June 11, 2012); the absence of *Zea perennis* was confirmed with herbarium curators (Nelson and Damrel, 2012). Isolated plants of teosinte identified as *Zea mays* ssp *parviglumis* var. *parviglumis* were collected in 1975 in Miami-Dade County, Florida (FLAS accession numbers 121428-121430) (Wunderlin and Hansen, 2008a) in an area that is now urban, and *Z. luxurians* has been reported as cultivated as a fodder in tropical Florida in the early 1900s (Taba, 1995). Experts familiar with the teosinte collections in the United States (T. Chochrane, University of Wisconsin-Madison Herbarium; M. Barkworth, Utah State University; and J. Doebley, and H. Iltis, University of Wisconsin, personal communication, June, 2012) (Barkworth, 2012; Cochrane, 2012) some of whom are currently involved with revision of the *Manual of Grasses for North America,* are not aware of any naturalized or native populations of teosinte currently growing in the United States.

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three (*T. dactyloides, T. floridatum*, and *T. lanceolatum*) exist as wild and/or cultivated species in the continental U.S. (OECD, 2003), and two taxa (*T. fasciculatum* and *T. latifolium*) also occur in Puerto Rico) (USDA-NRCS, 2011). Though many of these species occur where corn might be cultivated, gene introgression from HCEM485 corn under natural conditions is highly unlikely. In contrast with corn and teosinte, which may hybridize relatively easily under certain conditions, special techniques are required to hybridize corn and *Tripsacum* thereby making hybrids of *Tripsacum* species with *Zea* difficult to obtain outside of a laboratory; and offspring are often sterile or have greatly reduced fertility, and are unable to withstand even mild winter conditions (Mangelsdorf, 1974; Russell and Hallauer, 1980; Galinat, 1988). Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm et al., 1979).

Introgression of genes from corn into teosinte or *Tripsacum* species in the U.S. has not been described to occur in nature. While some teosinte may be considered weeds in certain instances, they are also used by some farmers for breeding improved corn (Sánchez and Ruiz, 1997). Teosinte is described as being susceptible to many of the same pests and diseases that attack cultivated corn. In the wild, introgressive hybridization from corn to teosinte is currently limited by several factors including geographic isolation, differing degrees of genetic incompatibility, differences in flowering time, developmental morphology, dissemination, and dormancy (Galinat, 1988; Doebley, 1990a; Doebley, 1990b; Ellstrand et al., 2007). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity, which serves as a significant constraint to introgression. Guadagnuolo et al. (2006) did report that hybrids between a glyphosate-resistant maize cultivar and "chalco teosinte" (*Z. mays* ssp. *mexicana*) showed greater vigor and produced more seeds than the wild parent. Nonetheless, in the absence of selective pressure from glyphosate there was no direct positive or negative impact of the transgene on the fitness or vigor of either the hybrids or the pure maize progeny. There have been no reports that the widespread cultivation of glyphosate resistant corn in the United States has resulted in the introgression of the engineered trait into related species in the field; no related species are listed as glyphosate resistant (Heap, 2012).

Data included in the Petition (Stine 2011, page 31, Table 11 and Figure 12) demonstrated that there were no discernible differences between HCEM485 corn and control corn in the absence of glyphosate treatment in terms of mean pollen diameter and overall pollen morphology. The petitioner did find a statistical difference in pollen viability between HCEM485 and control plants, though the values for both corn varieties were still within the normal range of corn pollen reference samples and the out-crossing rate of HCEM485 corn will likewise not be different compared to other corn varieties. Based on the data presented in the Petition, HCEM485 corn does not exhibit characteristics that cause it to be any weedier than other cultivated corn; and the extremely limited potential for gene introgression from HCEM485 corn into teosinte or *Tripsacum* species is not expected to be any different than that of other cultivated corn varieties. Even in the unlikely event that gene flow were to occur between HCEM485 corn and wild relatives, there is no reason to expect that the possible expression of modified EPSPS would transform corn wild relatives into more weedy species, especially in the absence of herbicide selection pressure. Hybrids, if they occurred, could possibly have diminished susceptibility to glyphosate herbicides if the gene encoding the double mutated 2mEPSPS is inherited and expressed in the hybrid, but the hybrids would still be susceptible to many other herbicides, as is HCEM485, and as are the many other glyphosate resistant corn varieties that have been available for approximately 15 years.

In summary, HCEM485 corn, like the antecedent G21, will not adversely impact sexually compatible wild relatives or the weediness attributes of those wild relatives.

Potential Impacts of HCEM485 on Organisms Considered Beneficial to Agriculture

HCEM485 is agronomically and compositionally similar to GA21 and other corn varieties in commercial production and therefore will not adversely affect other organisms in any new or novel way. In particular, with respect to the secondary metabolites and antinutrients (ferulic acid, ρ-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor) analyzed in grain samples from HCEM485 and control hybrids (Stine 2011, Table 19, page 41), the only significant difference noted was a small increase (*ca.* 3.4%) in trypsin protease inhibitor in HCEM485 grain samples, but this difference was not consistent across all growing locations and the level is well within the range reported in the literature for corn. APHIS has found no evidence or reason to believe that any differences between HCEM485 and GA21, or between HCEM485 and other corn varieties, in either the genetic material present or the proteins produced, would have any impact on other organisms, including organisms beneficial to agriculture (such as earthworms, honeybees, predators or parasites of corn pests). The EPSPS protein is expressed in a variety of plant tissues in corn, and expression of 2mEPSPS in HCEM485 corn is expected to be expressed in the same tissues since it is driven by the same regulatory sequences as the native glyphosate-sensitive EPSPS gene. Limited data from western blot analysis provided in the petition (Stine 2011, Figure 8, page 20) demonstrated that the level of expression of 2mEPSPS in leaf and seed of HCEM485 is less than that observed in the antecedent organism GA21. The inserted genetic material is not secreted, is not toxic, and does not produce any substance that is secreted or that would be considered toxic. The safety of modified EPSPS proteins from both plant and bacterial sources has been previously addressed in other petitions for deregulation (e.g. Monsanto submitted petitions 97-099-01p for GA21 corn and 00-011-01p for NK603 corn) and by the OECD (1999)). The EPSPS protein, both modified and unmodified, does not share meaningful amino acid sequence similarities with known toxins and is already present in a large portion of corn that is present in the food supply chain.

Potential to Transfer Genetic Information from HCEM485 Corn to Organisms with which it cannot Interbreed

APHIS examined the potential for the genetic material inserted into HCEM485 corn to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of more virulent plant pests. The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields in the bio-sciences since 1940, 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 was recently reviewed (Keese, 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake, recombination, and rearrangement, that occur most notably through viruses and mobile genetic elements. HGT has been implicated as a contributor to major transitions in evolution, including the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes, and viruses.

Potential for Horizontal Gene Transfer to Bacteria or Fungi

HGT from a plant species to other bacterial species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), there are almost no evolutionary examples of HGT to bacteria from eukaryotes or from plants to fungi (as reviewed in Keese 2008). The only genes likely to be transferred successfully from genetically engineered plants to bacteria are other bacterial genes. Horizontal transfer of the gene construct from the nuclear genome of HCEM485 corn and subsequent expression in bacteria is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and Rhizobium (Kaneko et al., 2000; Wood et al., 2001; Kaneko, 2002). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that HGT occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al., 2001; Brown, 2003). Third, the 6000-base pair insertion made to confer the herbicide resistance to HCEM485 corn is a small fraction of a percent (approximately 0.00024%) of the total DNA (approximately 2500 million base pairs) found in the corn genome, and all of the 6000 base pairs were derived directly from the corn genome. Forth, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression so even if HGT occurred, proteins corresponding to the transgenes are not likely to be produced. Finally, the FDA evaluated HGT from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA, 1998).

Potential for Horizontal Gene Transfer to Viruses

APHIS also considered whether horizontal transfer of DNA from HCEM485 corn to plant viruses was likely to occur and would lead to the creation or selection of a more virulent plant pathogen through recombination with other plant viruses. This issue has been considered before by other science review panels and government regulatory bodies (for a general review of the issue see (Keese 2008). Because there are no virus sequences used as part of the inserted gene cassette and no sequences that have been implicated in viral recombination or pathogenicity, there is no reason to think that viral recombination of the herbicide-resistance conferring genetic material will occur, or that such theoretical recombination would have any impact on plant pest risk.

Potential for Horizontal Gene Transfer to Parasitic Plants

Recently, Yoshida et al. (2010) through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*), which infests cereal fields (monocots) including corn and sorghum (*Sorghum bicolor*). According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed before speciation of purple witchweed (*S. hermonthica*) and related cowpea witchweed (*S. gesnerioides*), a parasitic plant of dicots, from their common ancestor. In other words, HGT between a parasitic plant and its host is an extremely rare event, and 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, 2011).

For all the reasons listed above, APHIS concludes that as with GA21 corn, HGT from HCEM485 corn to other organisms is unlikely to occur and thus poses no significant environmental or plant pest risk.

Conclusion

APHIS has reviewed and conducted a plant pest risk assessment on glyphosate-resistant HCEM485 corn. Due to similarities to the antecedent glyphosate-resistant GA21corn including the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of HCEM485 corn, the lack of atypical responses of HCEM485 corn to disease or plant pests in the field, the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem, and the lack of concerns of horizontal gene transfer, APHIS concludes that HCEM485 corn is unlikely to pose a plant pest risk.

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