Plant Pest Risk Assessment for Glyphosate Tolerant Sugar Beet Event H7-1 Grown for Root Production Under Certain Mandatory Conditions Imposed by APHIS

Background

In August 2010, APHIS received a supplemental request (dated July 29, 2010) from Monsanto Company (St. Louis, MO) and KWS SAAT AG (Einbeck, Germany) (Reding 2010) to amend their petition for nonregulated status submitted in 2003 (Petition 03-323-01p). The petitioner has requested that APHIS grant "partial deregulation" or similar administrative action to authorize the continued cultivation of sugar beet H7-1 subject to the interim measures proposed by APHIS in the lawsuit challenging its determination of nonregulated status of event H7-1 sugar beet. Responding to this request for "partial deregulation," this plant pest risk assessment was conducted to determine whether H7-1 sugar beet, when grown for root production under certain conditions (described briefly here, in the Environmental Assessment (EA) and in compliance agreements), is likely to pose a greater plant pest risk than the unmodified organism from which it was derived.

This plant pest risk assessment is limited in scope in that it only assesses the plant pest risks of H7-1 sugar beet root crop under mandatory conditions described in the EA and compliance agreements. The mandatory conditions apply to the interstate movement or importation of H7-1 sugar beet seed when destined for commercial root production, the planting and subsequent environmental release associated with growing H7-1 for root production, and the interstate movement of H7-1 sugar beets to processing facilities for processing into sugar and any associated by-products. This assessment does not reach a conclusion on the plant pest risk of a full deregulation of H7-1 sugar beets. Any action that APHIS may take in response to this request for "partial deregulation" would be an interim action, which is limited in scope and duration pending completion of a court-ordered EIS (noted and described below).

In 2003, Monsanto Company and KWS SAAT AG (hereafter KWS), petitioned APHIS (APHIS number 03-323-01p) for a determination that genetically engineered sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) event H7-1 is unlikely to pose a plant pest[1] 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 Protection Act (PPA) of 2000. Upon

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[1] The PPA defines a 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.

7 U.S.C. §7702(14).

completing a plant pest risk assessment, an Environmental Assessment (EA) and issuing a Finding of No Significant Impact (FONSI) (70 FR 13007-13008, Docket No. 04-075-2), APHIS advised the public of its determination, effective March 4, 2005, that the Monsanto/KWS sugar beet event H7-1 did not pose a plant pest risk and therefore was no longer considered a regulated article under APHIS regulations in 7 CFR part 340. Pursuant to that determination, sugar beet H7-1 was fully deregulated and no longer subject to any restrictions under the Plant Protection Act or 7 CFR part 340.

A complaint was filed in January, 2008, challenging APHIS' decision to grant nonregulated status to sugar beet event H7-1. On September 21, 2009, the US District Court for the Northern District of California found that APHIS should have prepared an environmental impact statement (EIS) as part of its decision making process to determine whether or not to grant nonregulated status (*Center for Food Safety et al. vs. Thomas Vilsack et al.*). On August 13, 2010, the Court vacated APHIS's decision to grant nonregulated status to sugar beet H7-1, making them subject to the Plant Protection Act of 2000 (PPA) and 7 CFR part 340 once again, and remanded the matter back to the agency to determine regulatory actions, if any, that should be imposed upon H7-1 sugar beet until the completion of the EIS.

In its plant pest risk assessments, APHIS considers 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, and transfer of genetic information to organisms with which it cannot interbreed. In conducting this plant pest risk assessment for partial deregulation, APHIS assessed the same type of information to determine whether there is a plant pest risk when grown under the mandatory conditions described in the EA and compliance agreement.

The mandatory restrictions and conditions related to growing the root crop are described in detail in a compliance agreement that will be required of all those who intend to import, move interstate, and/or release H7-1 sugar beet into the environment for commercial root production. Those desiring to enter into a compliance agreement must make their request to APHIS-BRS prior to conducting any of the noted activities. Information required by APHIS-BRS includes identification of the responsible party, contact information, location of the proposed environmental releases, the total proposed number of acres to be planted, and the origins and destinations of seed and/or roots being moved. The environmental release of H7-1 is limited to 11 states (noted in the EA and FONSI) and limited to sites which have been in agricultural production for a minimum of 3 years. APHIS-BRS will require both third party inspections as well as third party audits for all those parties with compliance agreements. APHIS-BRS will also conduct both inspections and audits to ensure compliance with all conditions of the compliance agreements. Planting of H7-1 sugar beets is prohibited in the state of California as well as a number of counties in Washington State. Growers are required to survey fields and remove sugar beet bolters as they are identified, records regarding these activities must be kept and these records are subject to audit to verify compliance. During transport of sugar beet seeds and roots, chain of custody documents must be maintained. Fields must be monitored for 3 years following harvest and any volunteer plants that are identified must be destroyed. Additionally, root crop growers must ensure that all field personnel are trained in the processes and procedures needed to comply with the compliance agreement. Those moving seed are required to ship their seeds in specific ways that minimize the likelihood of loss during shipment. Those moving roots for processing are required to load their vehicles in a manner that minimizes loss of beets during transport. Documentation of all these processes and procedures are required and are subject to audit by APHIS-BRS or an authorized third party inspector.

In this plant pest risk assessment, APHIS evaluated the gene inserted into H7-1 to determine if it causes plant disease. In addition, APHIS analyzed morphological characteristics of this sugar beet line to determine if this variety is likely to become weedy or invasive. Gene flow and introgression of the inserted genes into weedy and wild relatives, as well as related *Beta* species, was evaluated to determine the potential for increased weedy or invasive characteristics. Additionally, the likelihood of transfer of genetic information to organisms with which sugar beet cannot interbreed (horizontal gene transfer) was assessed. Finally, APHIS evaluated and compared H7-1 to conventional sugar beet with regard to disease and pest susceptibility. An assessment of the effects of the determination on non-target and beneficial organisms, and threatened and endangered species is included in the Final EA.

History of Development of H7-1 Glyphosate Tolerant[2] Sugar Beet

H7-1 is a genetically engineered sugar beet line that was developed to increase tolerance to the herbicide glyphosate. Glyphosate was first introduced as an herbicide under the trade name of Roundup® by Monsanto in 1975. Glyphosate is a systemic, post-emergence herbicide widely used on both agricultural commodities (food uses) and non-agricultural sites (Cerdeira 2006).

The management of weeds in sugar beet fields can be an expensive, labor intensive, and sometimes complicated operation. Often farmers use pre-emergent herbicides that will stop weed seeds from germinating. However, this assumes that weeds will always be a problem in all parts of a field. With H7-1, growers have the option of applying herbicide after weeds have germinated and only in areas of a field where weeds are present. Glyphosate is one of the most environmentally benign herbicides.

H7-1 sugar beets were genetically engineered to be glyphosate tolerant by inserting a gene (from *Agrobacterium* sp. strain CP4) that codes for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) into the sugar beet genome. This gene, along with its regulatory sequences, was introduced into these sugar beets via an *Agrobacterium*-mediated transformation protocol. This is a well-characterized procedure that has been widely used for decades to introduce various genes of interest directly into plant genomes.

APHIS authorized field testing of these sugar beets under permit and/or notification from 1998 through 2001. Event H7-1 sugar beets have been evaluated extensively for their agronomic characteristics and whether they present a plant pest risk. The field tests had been conducted in

[2] The applicant has described H7-1 sugar beet as "herbicide tolerant" and historically APHIS has also referred to GE plants with diminished herbicide sensitivity as "herbicide tolerant". However, the phenotype would fall under the Weed Science Society of America's (WSSA) definition of "herbicide resistance" since H7-1 has an inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type variety (WSSA 1998). By the WSSA definition, "resistance [to an herbicide] may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability" to survive and reproduce after herbicide treatment.

agricultural settings under physical and reproductive confinement conditions. After deregulation in 2005, H7-1 sugar beets were grown extensively for several growing seasons on hundreds of thousands of acres in the U.S.

Description of the Modification—Genetic material inserted and protein produced

Sugar beet H7-1 was produced by transformation using disarmed *Agrobacterium tumefaciens*. Sugar beet line 3S0057 (KWS proprietary line) cotyledons were infected with *Agrobacterium* strain CP4 containing plasmid PV-BVGT08. Plants containing the introduced DNA were selected based on growth in the presence of glyphosate.

The plasmid PV-BVGT08 contains a single expression cassette flanked by the right and left border sequences from the *Agrobacterium* Ti plasmid.

The gene cassette introduced into H7-1 sugar beet includes the following:

- The 35S promoter from a figwort mosaic virus (Gowda 1989, Sanger 1990) that is constitutively active in plants (Sheperd 1987, Richins 1987, Gowda 1989, Sanger 1990).

- The *ctp2* N-terminal chloroplast transit peptide (CTP) sequence from the *Arabidopsis thaliana epsps* coding region (Timko 1988) that targets the protein to the chloroplast.

- The *epsps* gene from *Agrobacterium* sp. strain CP4 (Padgette 1995) which has been sequenced and encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids (Padgette 1996).

- A 3' untranslated region (transcriptional terminator and polyadenlation site) of the *rbc*S E9 gene from *Pisum sativum* (Coruzzi 1984, Morelli 1985).

Data was provided and reviewed by APHIS that demonstrates stable integration and inheritance of the *epsps* gene and its associated regulatory sequences over several breeding generations (Petition 03-323-01p). Statistical analyses show that glyphosate tolerance is inherited as a dominant trait in a typical Mendelian manner. The enzyme EPSPS that confers glyphosate tolerance is from the bacterium *Agrobacterium* sp. strain CP4. This gene is similar to the gene that is normally present in sugar beets and is not known to have any toxic properties. This is the same gene and the same protein that is found in glyphosate tolerant corn, soybeans, and cotton that are grown on tens of millions of acres each year in the U.S.

Potential for H7-1 Sugar Beet to Become Invasive and/or a Weed

APHIS assessed whether the H7-1 sugar beet root crop grown under mandatory conditions described in the EA and compliance agreement is any more likely to become a weed than the non-transgenic recipient sugar beet line, or other sugar beets currently cultivated. The assessment encompasses consideration of the basic biology of sugar beet and an evaluation of unique characteristics of H7-1.

The parent plant in this petition, *Beta vulgaris* L. ssp. *vulgaris*, is not listed in the Weed Science Society of America's (WSSA) list of 3,488 weeds (WSSA 2010). In fact, no *Beta* species are included among the 1,553 weeds in the USDA database of invasive and noxious weeds (USDA 2010).

Sugar beets possess few of the characteristics of plants that are notable of successful weeds (Baker 1965; Keeler 1989). Occasionally, sugar beets volunteer in fields the year after harvesting. These plants can be controlled by mechanical means or the use of several other registered herbicides (Crop Protection Chemical Reference 1996). One of the mandatory conditions required by the partial deregulation is that growers monitor their fields for bolters, remove bolters if discovered, and keep records of bolters for APHIS' and/or third party inspection. Growers must also monitor their fields for volunteers for a three year period after harvest.

In trials conducted in both the U.S. and Europe, no differences were observed between H7-1 lines and non-transgenic lines with respect to the plant's ability to persist or compete as a weed. APHIS considered data relating to plant vigor, bolting, seedling emergence, seed germination, seed dormancy and other characteristics that might relate to increased weediness. No unusual characteristics were noted that would suggest increased weediness of H7-1 plants. Additionally, no characteristics relating to disease or insect resistance that might affect weediness were noted that were consistent over all trial locations. H7-1 sugar beet is still susceptible to the typical insect and disease pests of sugar beet. There was no indication from the data submitted that H7-1 possesses a selective advantage that would result in increased weediness. Therefore, H7-1 lacks the ability to persist as a troublesome weed, especially when grown with the requirement that bolters be removed, and there would be no direct impact on current weed management practices for sugar beet cultivation when grown for root production under mandatory conditions as described in the Final EA accompanying this risk assessment and in compliance agreements. Therefore, there is no selective advantage to sugar beet containing EPSPS compared to conventional sugar beet, and there is no increased potential for weediness or invasiveness from H7-1 sugar beet when grown for root production under the described mandatory conditions. Further, APHIS is not aware of any increased weedy characteristics of H7-1 while being grown under permit or notification prior to full deregulation or while being grown commercially on hundreds of thousands of acres after deregulation.

Potential for Gene Flow and Gene Introgression from H7-1into Sexually-Compatible Relatives

APHIS evaluated the potential for gene introgression to occur from the H7-1 root crop grown under mandatory conditions to sexually compatible relatives and considered whether such introgression would result in increased weediness. Sugar beets are sexually compatible with several other *Beta* species (OECD 2001). Under the mandatory conditions of the partial deregulation, the H7-1 sugar beet root crop will be prohibited from being grown in counties in Washington State where its sexually compatible relatives, Swiss chard and table beets, are grown for and/or produce seed and in California where its sexually compatible relatives may produce seed. The centers of origin for *Beta vulgaris* is generally believed to be in the Mediterranean or Near East region and no *Beta* species are known to be native to the U.S. (OECD 2001).

Although sugar beets have escaped cultivation and their progeny have persisted in the environment for many years (especially in California), these plants are not serious weed problems (Johnson 1958; Panella 2003). Some of these plants are found in the San Francisco Bay area where sugar beets are no longer cultivated. Another population of sexually compatible plants is in the Imperial Valley of California. The movement of the glyphosate tolerance trait from H7-1 to any other sexually compatible *B. vulgaris* should not have a significant impact especially if glyphosate is not applied to these plants. APHIS cannot find any evidence that herbicides are applied routinely to these plants living outside cultivated areas. Even if these plants become tolerant to glyphosate there are other registered herbicides that can be used to kill them and other methods of control can still be used (OECD 2001). APHIS concludes that the potential for gene flow to other sexually compatible *Beta* species is near zero, particularly when grown for root production under mandatory conditions described in the EA and Compliance Agreements. This is for two reasons. Under the mandatory compliance agreements, commercial root growers are required to scout for and remove bolters, thus not allowing pollen to be produced. Also, based on the mandatory restrictions and conditions of the compliance agreements, H7-1 is prohibited from being grown in California where other sexually compatible *Beta* species are known to exist. Further, H7-1 for root production is also prohibited from being grown in various counties in Washington State where Swiss chard and/or table beets are grown for seed. Further consideration and discussion of the potential for introgression of genes from sugar beet into related species can be found in the Appendix attached to this document.

Potential for Transfer of Genetic Information to Organisms with which H7-1 Cannot Interbreed

Horizontal gene transfer (HGT) 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 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).
(As shown by all the references noted in this section, HGT from plants to microbes is an extremely rare event and in the context of environmental release of H7-1 sugar beets, is extremely unlikely to occur. Even if it were likely for HGT to occur, movement of an *epsps* gene to a microbe would not provide a selective advantage to that microbe that is likely to have any novel or significant environmental impact. APHIS concludes, therefore, that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

Potential for H7-1 to have Altered Disease and Pest Susceptibilities

APHIS assessed whether the H7-1 root crop is likely to have significantly altered disease and pest susceptibility. This assessment encompasses consideration of the introduced trait and disease and pest susceptibility data from H7-1 releases into the environment.

Sugar beet is not a plant pest in the U.S. (http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist2006.pdf). The *Agrobacterium* transformed plants used in the generation of H7-1 were treated with an antibiotic to kill the *Agrobacterium* cells. Furthermore, DNA sequences derived from plant pests that were incorporated in H7-1 do not result in the production of infectious agents or disease symptoms in plants, and so it is unlikely that H7-1 could pose a plant pest risk. The description of the genetic modifications, including genetic elements, expression of the gene product and their functions for H7-1 has been summarized above.

Both qualitative and quantitative data addressing disease susceptibility, insect damage and overall agronomic performance were collected in order to assess possible effects from introduction of the *epsps* gene and its associated regulatory sequences. H7-1 was field tested in the U.S. over four years (1998-2001) at ninety-eight field trial sites representing a wide range of environmental conditions where sugar beets are commercially cultivated (Petition 03-323-01p). Plant disease was noted in thirty-six of the ninety-eight trial sites. Susceptibility to powdery mildew (*Erysiphe betae*), *Cercospora* leaf spot (*Cercospora beticola*), *Rhizoctonia* root rot (*Rhizoctonia solani*), fungal seedling disease (including *Pythium*, *Rhizoctonia*, and *Aphanomyces*, curly top virus and *Rhizomania* (Beet Necrotic Yellow Vein Virus – BNYVV) was similar in all but six field trials sites. Observations found reduced susceptibility to powdery mildews at three trial sites, increased susceptibility at one site, and no difference at nine sites where the disease was present. A slight increased susceptibility (10%) to *Cercospora* leaf spot was noted in two sites, while no differences were identified at eleven sites where *Cercospora* leaf spot was noted. These differing levels of resistance and susceptibility observed between H7-1 and comparator plants are likely due to the genetic backgrounds in the H7-1 plants, because there were no trends noted when differences were observed. European trials conducted in Germany and France over 2 years, using regionally adapted conventional sugar beet lines and genetically similar lines to H7-1, noted no differences in susceptibility to 10 different sugar beet pests (Petition 03-323-01p).

Nursery trials tested the performance of different plant varieties including H7-1 when challenged (artificially or natural infection) with plant pathogens (Petition 03-323-01p). During the 2000 and 2001 growing seasons, sugar beet nursery trials were conducted with H7-1 and conventional sugar beet varieties to assess disease resistance to *Cercospora* leaf spot, *Aphanomyces* root rot, curly top virus and *Rhizoctonia* root rot. H7-1 infection levels of these diseases were found to be within the range of ratings observed for the conventional registered varieties. Greenhouse trials using *Fusarium* and *Rhizoctonia* isolates have found that some of the isolates produced greater disease severity on H7-1 treated with glyphosate (Hanson 2003; Larson 2006). Other researchers have suggested that it may be difficult to predict field results from greenhouse/laboratory experiments (Estok 1989; Wan 1998). Subsequent field studies have not shown increased incidence of these diseases (Khan 2010).

The major insect and nematode pests in the U.S. (sugar beet root aphid (*Pemphigus populivenae*), sugar beet root maggot (*Tetanops myopaeformis*), sugar beet cyst nematode (*Heterodera schachtii*) and root knot nematode (*Meloidogyne arenaria, M. incognitata, M. javanica* and *M. hapla*) were monitored during the U.S. field trials. No observed differences were observed in any of the 98 field trials.

Al-Kaff (1998) noted gene silencing effects when transgenic plants have been infected by a virus with DNA sequence homology to a portion of the introduced genes. None of the viral diseases of beet is related to figwort mosaic virus (http://image.fs.uidaho.edu/vide/refs.htm and Whitney 1986) (a caulimovirus and from which the promoter for the *epsps* gene originates) so silencing of the *epsps* gene should not occur.

In addition to field studies on agronomic parameters, Monsanto/KWS analyzed sugar beets for compositional changes as part of their submission to FDA in the consultation process (FDA 2004). While FDA uses these data as indicators of possible nutritional changes, APHIS views them as a general indicator of possible unintended changes. Compositional analyses evaluating carbohydrates, proteins, fiber, fat, sugars and eighteen amino acids (a total of 55 statistical comparisons) identified seven statistically different values compared with the near isogenic control line. All values, however, fell within the range of values observed for both the near isogenic control line and conventional sugar beet varieties, providing additional evidence that event H7-1 sugar beet does not exhibit unexpected or unintended effects.

Other phenotypic characterizations comparing H7-1 lines with conventional and control lines were also completed. Data was provided and assessed by APHIS on leaf color, leaf size, hypocotyl color, seed germination and dormancy, vernalization, bolting, flowering onset and seed harvest date. No qualitative or quantitative observations indicated any biologically meaningful differences from control lines or differences outside the range of conventional sugar beet norms.

Given the interactions between the environment, the genetic backgrounds of the cultivars used and some inherent genetic variability within sugar beet varieties, APHIS concludes that these results regarding disease and pest susceptibilities are not unexpected and do not indicate an increased plant pest risk. Additionally, the pest and disease susceptibility of H7-1 sugar beets are innate characteristics of these plants. Finally, production of the EPSPS protein in event H7-1 sugar beet is not expected (1) to cause plant disease or influence susceptibility of H7-1 to diseases or pests and (2) plant pest effects on raw or processed plant commodities (e.g., sugar, beet pulp, etc) are highly unlikely.

Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if H7-1 sugar beet, when grown for root production under mandatory conditions described in compliance agreements, is likely to pose a plant pest risk. Based on the information provided by the applicant and the lack of any identified plant pest risk from the inserted genetic material, weedy characteristics, atypical responses to disease or plant pests in the field, and horizontal gene transfer, APHIS has concluded that H7-1 sugar beets, when grown for root production under mandatory conditions described in the Environmental Assessment and compliance agreements, are unlikely to pose a plant pest risk.

Based on APHIS's analysis of field, greenhouse, and laboratory data and references provided in the petition, amended petition, accompanying environmental data, and other relevant information as cited, APHIS concludes that H7-1 sugar beets, when grown for commercial root production under mandatory conditions described in compliance agreements (described in detail in the EA), are unlikely to pose a plant pest risk for the following reasons:

(1) They exhibit no plant pathogenic properties. Although a plant pathogen was used in their development, these plants are not infected by this organism nor do they contain genetic material from pathogens used as a donor organism that can cause plant disease. No new protein other than the intended CP4 EPSPS was produced and there was no unintended change in the genome of H7-1 sugar beet as a result of the insertion.

(2) They exhibit no characteristics that would cause them to be weedier than the non-transgenic parent sugar beets or other cultivated sugar beets and several control options besides glyphosate are available for control of feral or volunteer plants. The mandatory conditions contained in the compliance agreements require that growers monitor their fields for volunteers and destroy any that are found for a three year period after harvest.

(3) Gene flow and introgression from H7-1 root crop to introduced or naturalized *Beta* species in the United States, when grown under mandatory conditions contained in compliance agreements, is extremely unlikely and even if it were to occur, is not likely to increase the weediness potential of any resulting progeny any more than would introgression from other cultivated *Beta* species. Prohibitions on growing H7-1 in several counties in Washington State where sexually compatible relatives, Swiss chard and table beets, are grown for and/or produce seed and in California where its sexually compatible relatives may produce seed, preclude this from occurring. Additionally, removal of bolters in commercial fields, as required under the mandatory compliance agreements, eliminates the possibility of pollen production.

(4) Horizontal gene transfer from the H7-1 root crop is highly unlikely to occur, and is not expected to pose a plant pest risk.

(5) Disease and pest susceptibility and compositional profiles of H7-1 are similar to those of the parent variety and other sugar beet cultivars grown in the United States; therefore, pest and disease control methods are expected to be similar and no direct or indirect plant pest effect on raw or processed plant commodity is expected.

As noted previously, this plant pest risk assessment is limited in scope in that it only assesses the interstate movement or importation of H7-1 sugar beet seed when destined for commercial root production, the planting and subsequent environmental release associated with growing H7-1 for root production, and the interstate movement of H7-1 sugar beets to processing facilities for processing into sugar and any associated by-products. This assessment is also predicated on the assumption that all persons involved in the noted activities will comply with mandated conditions contained in mandatory compliance agreements. USDA/APHIS anticipates that a more comprehensive plant pest risk assessment describing its assessment of environmental release of H7-1 sugar beets without conditions, a full deregulation of the seed crop and commercial root crop, will be prepared in conjunction with the Environmental Impact Statement that is under development by the Agency.

References

Al-Kaff, N.S., Covey, S.N., Kreike, M.M., Page, A.M., Pinder, R., Dale, P.J. (1998) Transcriptional and Posttranscriptional Plant Gene Silencing in Response to a Pathogen. Science 279: 2113-2115

Baker, H.G. (1965) Characteristics and Modes of Origin of Weeds. *In*: The Genetics of Colonizing Species. pp. 147-172. Baker, H. G., Stebbins, G. L. (eds.). Academic Press, New York and London

Brown, J.R. (2003) Ancient horizontal gene transfer. Nature Review Genetics 4:121-132

Cerdeira, A.L., Duke, S.O. (2006) The Current Status and Environmental Impacts of Glyphosate-Resistant Crops: A Review. Journal of Environmental Quality 35:1633-1658

Crop Protection Chemical Reference. (1996) Chemical and Pharmaceutical Press, New York.

Coruzzi, G., Broglie, R., Edwards, C., Chua, N. (1984) Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. EMBO J. 3:1671-1679

Estok, D., Freedman, B., Boyle, D. (1989) Effects of the herbicides 2,4-D, glyphosate, Hexazinone, and Triclopyr on the growth of three species of ectomycorrhizal fungi. Bull. Environ. Contam. Toxicol. 42:835-839

FDA (1998) Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants. pp. 28

FDA (2004) Completed consultations on bioengineered foods. United States Food and Drug Administration. BNF No. 90

Gowda, S., Wu, F.C., Sheperd, R.J. (1989) Identification of promoter sequences for the major RNA transcripts of figwort mosaic and peanut chlorotic streak viruses (caulimovirus group). J. Cell. Biochem 13D (supplement):301

Hanson, L, (2003) Effect of treating sugar beet with the herbicide glyphosate on *Fusarium* yellows. Proceeding of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. Published Sept. 2, 2003

Johnson, R. T., Burtch, L.M. (1958) The problem of wild annual sugar beets in California. Jour. Am. Soc. of Sugar Beet Tech. 10(4): 311-317

Kaneko, T. *et al.* (2000) Complete genome structure of the nitrogen-fixing symbiotic 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

Keeler, K. (1989) Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139

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

Khan, M.F. (2010) Introduction of glyphosate-tolerant sugar beet in the United State. Outlooks on Pest Management 21:38-41

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

Larson, R.L., Hill, A.L., Fenwick, A., Kniss, A.R., Hanson, L.E., Miller, S.D. (2006) Influence of glyphosate on *Rhizoctonia* and *Fusarium* root rot in sugar beet. Pest Management Science 62:1182-1192

Morelli, G., Nagy, F., Fraley, R.T., Rogers, S.G., Chua, N.H. (1985) A short conserved sequence is involved in the light-inducibility of a gene encoding ribulose 1,5-bisphosphate carboxylase small subunit of pea. Nature 315:200-204

OECD (1999) Organization for Economic Cooperation and Development. Consensus document on general information concerning the genes and their enzymes that confer tolerance to glyphosate herbicide. OECD Environmental Health and Safety Publications. Paris. ENV/JM/MONO (99)9.

OECD (2001) Consensus Document on the biology of *Beta vulgaris* L. (Sugar Beet). In Series on Harmonization of Regulatory Oversight in Biotechnology, p 40

Padgette, S.R., Kolacz, K.H., Delannay, X., Re, D.B., Lavallee, B.J., Tinius, C.N., Rhodes, Y.I., Otero, W.K., Barry, G.F., Eichholtz, D.A., Peschke, V.M., Nida, D.L., Taylor, N.B., Kishore, G.M. (1995) Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Science 35:1451–1461

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T. (1996) New weed control opportunities: development of soybeans with a Roundup ready™ gene. Pp. 54-84. *In* Agricultural, Environmental, Econonmic, Regulatory, and Technical Aspects. S.O Duke, editor. CRC Press Inc. 4:54-84

Panella, L.(2003) USDA/ARS Research Geneticist and Sugarbeet Germplasm Committee Chair, Personal communication to J.R. Stander, Betaseed, Inc. (Appendix 2 of the Petition).

Reding, H.K., von dem Bussche, P., Hofmann, P. (2010) Letter to Michael C. Gregoire, USDA APHIS, July 29, 2010. Attached to Environmental Report submitted by Monsanto in support of petition 03-323-01p.

Richins, R.D., Scholthof, H.B., Sheperd, R.J. (1987) Sequence of figwort mosaic virus DNA (Caulimovirus Group). Nucl. Acids Res. 15:8451-8466

Sanger, M., Daubert, S., Goodman, R.M. (1990) Characteristics of a strong promoter from figwort mosaic virus: comparison with the analogous 35S promoter from cauliflower mosaic virus and the regulated mannopine synthase promoter. Plant Molecular Biology 14:433-443

Schneider, R.W., Strittmatter, G. (2003) Petition for Determination of Nonregulated Status for Roundup Ready ® Sugar Beet Event H7-1 http://www.aphis.usda.gov/brs/aphisdocs/03_32301p.pdf

Sheperd, R.J., Richins, J.F., Duffus, J.F., Handley, M.K. (1987) Figwort mosaic virus: properties of the virus and it adaptation to a new host. Phytopathology 77:1668-1673

Siciliano, S.D., Germida, J.J. (1999) Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the nontransgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. FEMS Microbiol. Ecol. 29:263-272

Timko, M.P., Herdies, L., de Alameida, E., Cashmore, A.R., Leemans, J., Krebbers, E. (1988) Genetic engineering of nuclear-encoded components of the photosynthetic apparatus of *Arabidopsis.* Pp. 279-295. *In* The Impact of Chemistry on Biotechnology – A Multidisciplinary Discussion. M. Phillips, S.P. Shoemaker, R.D. Middlekauff, R.M. Ottenbrite, editors. ACS Books, Washington, D.C.

USDA (2010) Invasive and noxious weeds. United States Department of Agriculture. Accessed on October 29, 2010 at: http://plants.usda.gov/java/invasiveOne?startChar=B

Wan, M.T., Rahe, J.E., Watts, R.G. (1998) A new technique for determining the sublethal toxicity of pesticides to the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. Environ. Toxicol. Chem. 17(7):14-21

Whitney, E.D., Duffus, J.E. (1986) Compendium of Beet Diseases and Insects. pp. 26-32

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

WSSA Weed Science Society of America (2010) Composite list of weeds as compiled by the standardized plant names subcommittee. Weed Science Society of America. Accessed on October 29, 2010 at: http://wssa.net/Weeds/ID/WeedNames/namesearch.php

Appendix: Sugar Beet Biology and the potential for introgression into related species: A Worldwide Assessment

Sugar beets, *B. vulgaris* L. ssp. *vulgaris*, are a member of the family Chenopodiaceae which also includes the leaf beet (Swiss chard) and the red table beet (fodder beet), from which the sugar beet was derived (Cooke 1993; OECD 2001). Members of this family are dicotyledonous and usually herbaceous in nature. Sugar beets are grown worldwide. Sugar beet is largely wind pollinated and is normally a biennial that develops a large succulent root the first year and a seed stalk the second. Since sugar beets are normally harvested during the first year while still in the vegetative phase, flowers rarely develop. However, certain conditions such as low temperatures after planting and longer day length can induce the sugar beet to "bolt" or produce a seed stalk during the first growing season (Bell 1946; Jaggard 1983; Durrant 1988).

The genus *Beta*, including wild relatives, is divided into four sections with various species and subspecies (Lange 1999; Frese 2001): Section *Beta* with *B. vulgaris* ssp. *vulgaris,* ssp. *maritima*, and ssp. *adanensis*, *B. macrocarpa,* and *B. patula*: Section Corollinae with *B. corolliflora*, *B. macrochiza*, *B. lomatogona*, *B. intermedia* and *B. trigyna,* and Section Procumbentes with *B. patellaris*, *B. procumbens, B. webbiana,* and Section Nanae with *B. nana*. Some researchers (Bartsch 1999; Bartsch 2003) consider *Beta macrocarpa* as a separate species; however, USDA ARS reports that the designation was changed in 2000 to *B. vulgaris* ssp*. macrocarpa* (USDA ARS 2010). There is some scientific disagreement about the compatibility of sugar beet and *B. vulgaris* ssp*. macrocarpa* (referred to hereafter as *B. macrocarpa*, the terminology in all sources except USDA ARS 2010).

Sugar beet hybridizes freely with all members of the section *Beta* and the resulting progeny are fully fertile. Hybrids between sugar beet and members of the other three sections do not naturally occur without human intervention. Artificial hybrids can be produced with difficulty with species in Section Corollinae; however, these hybrids are highly sterile and set few seeds when backcrossed to sugar beet. Hybrids between sugar beet and Section Procumbentes members normally die at the seedling stage. No hybrids between cultivated beets and *B. nana* have been reported. Therefore, natural crosses between cultivated sugar beet and species from Sections other than *Beta* are highly unlikely.

In the genus *Beta* there are different examples of weediness. For example, the species itself can be a serious weed. According to Holm (1979, 1991), *B. vulgaris* (subspecies not given) is classified as an occasional to serious weed in the following countries: Afghanistan, Australia, Mexico, Morocco, the United States, Iraq, Israel, Portugal, and Egypt. Subspecies *maritima* (wild sea beet) is a problem weed in coastal regions of the Mediterranean Sea and North Sea in Europe and in Asia. In other examples, natural hybrids between cultivated sugar beet and resident species have occurred to produce weed beets in commercial operations. For example, hybrids between *Beta macrocarpa* and commercial sugar beets are a weed problem in production fields (Hultén 1986) and natural hybrids have also occurred between cultivated sugar beets (*Beta vulgaris* ssp. *vulgaris*) and wild beets (*Beta vulgaris* ssp. *maritima*) in Europe. This has resulted in a hybrid form of "weed beet" that can bolt in a single season, while growing among biennial sugar beet varieties (Parker 1996). Weed beet populations are described as possessing domesticated characteristics such as wider leaves and an annual growth habit (Lange 1999). Weed beets cause yield losses and can delay harvest (Bartsch 2003).

Possible movement of the transgenes via pollen from event H7-1 to other members of the *Beta* section would be species and geographically specific. Movement of the transgenes to *B. vulgaris* ssp. *adanensis*, ssp. *maritima*, and B. *patula* is not likely in the United States since these plants are not found in the Americas. Based on a search for wild *Beta* populations in the United States, (USDA NRCS 2010) two species are known to occur. *Beta procumbens* occurs only in Pennsylvania and *Beta vulgaris* is known to have escaped from cultivation in some states (see Figure 1).

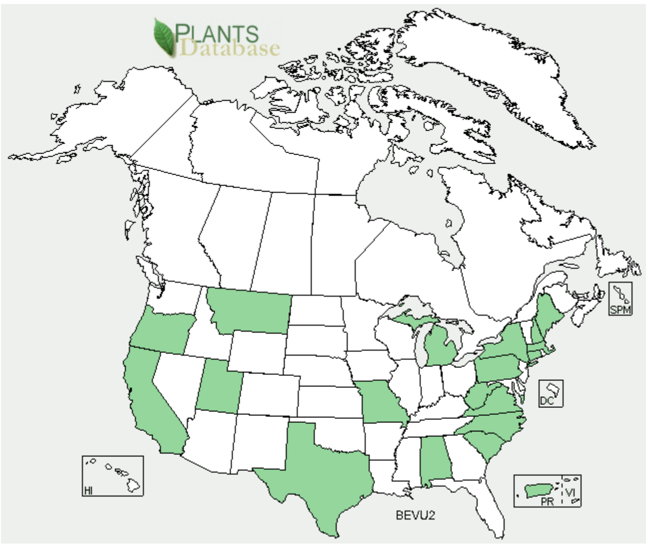

Figure 1. Distribution of *B. vulgaris* in the U.S. (USDA CRCS 2010)

The largest significant populations occur in California, a state where the H7-1 sugar beet root and seed crops will not be permitted to be grown. Wild beets are found from the San Francisco Bay area to the Mexican border (Bartsch 1999), concentrated mainly in the Bay area, and in the Imperial Valley. The Californian wild or weed beets belong to two different taxa, *B. vulgaris* and *B. macrocarpa*, and have at least three different origins (Bartsch 2002). They evolved from escaped Swiss chard or red beet, from *B. macrocarpa*, or from hybridization of *B. vulgaris* with introduced *B. macrocarpa.*

Hybridization of H7-1 with the wild *B. procumbens* in Pennsylvania is unlikely to occur due to species incompatibility (see above). In California, sugar beet plants, *B. vulgaris*, that escaped from past commercial cultivation in the San Francisco Bay area are unlikely to cross with H7-1 since sugar beets will not be permitted to be grown in California. Thus transgene movement via pollen to these plants is extremely unlikely.

Sugar beet production continues in the Imperial Valley and is a major center of production. There are free living sugar beets that have escaped cultivation and have persisted (Johnson 1958; McFarlane 1975) and these plants are a minor weed problem in this area. In addition *B. macrocarpa* species grow as a weed beet in sugar beet fields in this location (Bartsch 2002) and even though *B. macrocarpa* usually flowers earlier than sugar beet, it can cross with sugar beet bolters when flowering times overlap (Bartsch 2002). However, movement of the transgenes from H7-1 to these plants is not possible since H7-1 will not be grown in the Imperial Valley.

A number of scientists (Boudry 1993; Bartsch 1996; Desplanque 2002; Bartsch 2003) have studied the potential movement of herbicide tolerance genes from commercial sugar beets to sexually compatible relatives. Desplanque (2002) has shown that in France, when weed beets were present in variable densities in sugar beet fields, that the transfer of an herbicide resistance trait would be possible into the weed beet population, and suggested ways that this could be mitigated, for example by only incorporating the transgene for herbicide tolerance into tetraploid pollinator breeding lines. Additionally selecting cultivars that have a reduced tendency to bolt would reduce the likelihood of introgression of the trait into weed beet populations.

References

Bartsch, D., Pohl-Orf, M. (1996) Ecological aspects of transgenic sugar beet: Transfer and expression of herbicide resistance in hybrids with wild beets. Euphytica 9: 55-58

Bartsch, D., Ellstrand, N.C. (1999) Genetic evidence for the origin of Californian wild beets (genus *Beta*). Theoretical and Applied Genetics 99:1120-1130

Bartsch, D., Driessen, S., Gathmann, A., Hoffmann, A., Lehnen, M., Muecher, T., Saeglitz, C., Wehres, U., Schuphan, I. (2002) Monitoring the environmental consequences of gene flow from transgenic sugar beet. In Gene Flow Workshop, The Ohio State University, pp 78-93

Bartsch. D., Cuguen. J., Biancardi. E., Sweet, J. (2003) Environmental implications of gene flow from sugar beet to wild beet -- current status and future research needs. Environmental-Biosafety-Research 2: 105-115

Bell, G.D.H. (1946) Induced bolting and anthesis in sugar beet and the effect of selection of physiological types. Journal of Agricultural Science 36:167-184

Boudry, P., Moerchen, M., Saumitou-Laprade, P., Vernet, P., van Djik, H. (1993) The origin and evolution of weed beets: Consequence of the breeding and release of herbicide-resistant transgenic sugar beets. Theoretical and Applied Genetics 87:471-478

Cooke, D.A., Scott, R.K. (1993) The Sugar Beet Crop: Science Into Practice. Chapman & Hall, 675 p. New York

Desplanque, B., Hautekèete, N., Van Dijk, H. (2002) Transgenic weed beets: possible, probable, avoidable? Journal-of-Applied-Ecology 39:561-571

Durrant , M.J., Jaggard, K.W. (1988) Sugar beet seed advancement to increase establishment and decrease bolting. Journal of Agricultural Science 110:367-374

Frese, L. (2001) Sugar beets and related wild species - from collecting to utilisation. In H Knüpffer, J Ochsmann, eds, Rudolf Mansfeld and Plant Genetic Resources. Proceedings of a symposium dedicated to the 100th birthday of Rudolf Mansfeld. Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, D-06466, Gatersleben, Germany, Gatersleben, Germany, 8-9 October 2001, pp 170-181

Holm, L., Pancho, J.V., Herbarger, J.P., Plucknett, D.L. (1979) A geographical atlas of world weeds. John Wildy and Sons, New York

Holm, L., Pancho, J.V., Herbarger, J.P., Plucknett, D.L. (1991) A geographical atlas of world weeds. Kreiger Publ. Co., Malabar, FL

Hultén, E., Fries, M. (1986) Atlas of north European vascular plants north of the Tropic of Cancer. Koeltz Scientific, Königstein, Federal Republic of Germany

Jaggard, K.W., Wickens, R., Webb, J.D., Scott, R.K. (1983) Effects of sowing date on plant establishment and bolting and the influence of these factors on yields of sugar beet. Journal of Agricultural Science 101: 147-161

Johnson, R.T., Burtch, L.M. (1958) The problem of wild annual sugar beets in California. Journal of Agricultural Science 10: 311-317

Lange, W., Brandenburg, W.A., De Bock, T.S.M. (1999) Taxonomy and cultonomy of beet (*Beta vulgaris* L.). Botantical Journal of the Linnean Society 130:81-96

McFarlane, F.S. (1975) Naturally occurring hybrids between sugar beet and *Beta macrocarpa* in the Imperial Valley of California. Journal of American Society of Sugar Beet Technologists 18:245-251

OECD (2001) Consensus Document on the biology of *Beta vulgaris* L. (Sugar Beet). In Series on Harmonization of Regulatory Oversight in Biotechnology, p 40

Parker, I.M., Bartsch, D. (1996) Recent advances in ecological biosafety research on the risks of transgenic plants: a transcontinental perspective. In T Tomiuk, K Wöhrmann, A Sentker, eds, Transgenic organisms: Biological and Social Implications. Birkhause Verlag, Basel, pp 147-161

USDA ARS (2010) National genetic resources program germplasm resources information network - (GRIN) [Online Database]. United States Department of Agriculture, Agriculture Research Service. National Germplasm Resources Laboratory, Beltsville, Maryland. Accessed on October 29, 2010 at: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1441384

USDA NRCS (National Resources Conservation Service (2010) The PLANTS Database (http://plants.usda.gov,). National Plant Data Center, Baton Rouge, LA 70874-4490 USA. Accessed on October 25, 2010