Plant Pest Risk Assessment of Event H7-1 Sugar Beet

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A. Introduction

Monsanto Company (St. Louis, MO) and KWS SAAT AG (Einbeck, Germany), petitioned APHIS (APHIS number 03-323-01p) for a determination that genetically engineered (GE) sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) event H7-1 is unlikely to pose a plant pest risk (Monsanto and KWS SAAT AG 2004) and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000[1]. This plant pest risk assessment was conducted to determine if H7-1 is unlikely to pose a plant pest risk.

The initial request was received from Monsanto and KWS SAAT AG in 2003. Upon 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 and KWS SAAT AG (2004) 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. A complaint was filed in January, 2008, challenging APHIS' decision to grant nonregulated status to event H7-1. On September 21, 2009, the US District Court for the Northern District of California ruled that APHIS should have prepared an Environmental Impact Statement before making a decision to grant nonregulated status to event H7-1 (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 the event H7-1 sugar beet varieties 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 event H7-1 until the completion of the Final EIS and a new decision could be made by APHIS as to whether it would be appropriate to grant full nonregulated status to event H7-1.

APHIS received a supplemental request from Monsanto and KWS SAAT AG (2010) to amend the petition for non-regulated status submitted in 2003 (Petition 03-323-01p) pursuant to the regulatory scheme of 7 CFR part 340 (Reding 2010). These petitioners requested that APHIS grant "partial deregulation" or similar administrative action to authorize the continued cultivation of event H7-1 subject to the interim measures proposed by APHIS in the lawsuit challenging its 2005 determination of non-regulated status of event H7-1. On February 8, 2011, APHIS advised the public of its decision to "partially deregulate" event H7-1 sugar beets as an interim action until the court-ordered EIS was completed. That "partial deregulation" described a system in which root crop production of event H7-1 sugar beets could occur under a Compliance Agreement with USDA/APHIS and growing of event H7-1 for seed production could occur under APHIS' permitting system. The conditions under which commercial H7-1 root production is carried out are enforceable under the mandatory compliance agreements. A Plant Pest Risk Assessment for root production was prepared to consider the plant pest risk potential of H7-1 sugar beet root crop under the conditions of the interim action.

[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

H7-1 was grown in the U.S. under APHIS permits or notifications from 1998 through 2005. From 2005 to 2010, H7-1 was grown widely without APHIS regulatory restrictions. Since February 2011, H7-1 sugar beets have been grown commercially for root production under APHIS-mandated compliance agreements and for seed production under APHIS issued permits. This Plant Pest Risk Assessment was conducted to determine whether sugar beet event H7-1 is unlikely to pose a plant pest risk under full deregulation.

Sugar beet line H7-1 was produced by transformation of a sugar beet line (3S0057) with *Agrobacterium tumefaciens.* Because *A. tumefaciens* is a plant pest and some of the regulatory sequences (figwort mosaic virus promoter) used to facilitate expression of the gene and the sequences that code for the protein (*cp4 epsps* gene from *A. tumefaciens*) in sugar beet were derived from plant pests, H7-1 has been considered a regulated article under APHIS regulations at 7 CFR part 340. Potential impacts to be addressed in this risk assessment are those that pertain to the use of H7-1 and its progeny in the absence of confinement. APHIS utilizes data and information from current scientific literature, in addition to information submitted by the applicant, to determine if H7-1 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is not a plant pest, then APHIS has no regulatory authority over that organism. Thus, if APHIS determines that H7-1 sugar beet does not pose a plant pest risk, then H7-1 sugar beet would no longer be subject to the plant pest provisions of the Plant Protection Act or to the regulatory requirements of 7 CFR part 340, and therefore, APHIS must grant it non-regulated status.

Potential impacts analyzed in this Plant Pest Risk Assessment are those that pertain to plant pest risk characteristics. APHIS regulation 7 CFR 340.6(c) specifies the information a petitioner must include in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, potential for effect on non-target organisms, weediness of the regulated article, any impacts on the weediness of any other plant with which it can interbreed, potential changes to agriculture or cultivation practices and transfer of genetic information to organisms with which it cannot interbreed.

Pursuant to NEPA, the EIS for this petition for full deregulation considers whether agricultural or cultivation practices associated with H7-1 may result in impacts on the environment. A thorough assessment of H7-1's effects on the environment including on non-target and beneficial organisms, and threatened and endangered species is included in the Final EIS.

Sugar beets (*Beta vulgaris* (L.) ssp. *vulgaris*), a member of the genus *Beta* (Chenopodiaceae), are cultivated worldwide. The species *B. vulgaris* includes many different crop varieties including sugar beets, fodder beets, Swiss chard, and table beets. *Beta vularis* L. spp. *maritima* (L.) Arcang. wild sea beet, is regarded as the progenitor of the *Beta* crop species (fodder beet, sugar beet, Swiss chard and table or red beet) and found widely distributed over the Mediterranean basin and the Near East (OECD 2001).

Sugar beet is a biennial species (typically will complete its lifecycle in 2 years) that is grown as an annual for sugar production. The plant grows as a rosette in its first year and can be quite variable in size (from 1-6 ft. tall). If allowed to overwinter in a moderate climate, it will "bolt"

the second year and produce a flower stalk and seeds (OECD 2001). Being a biennial, the plant typically flowers during the late spring to summer of the 2nd year of growth, usually sending up a single unbranched flower stalk. Flowers begin to release pollen about 4-6 weeks after the flower stalk emerges and continue to release pollen for another 2-4 weeks. This biennial plant life cycle of reduced flowering in the first year has been selected for in sugar beets and vegetable beet varieties. Typically the level of bolting in cultivated sugar beet is reduced to 0.01 – 1 percent (Ingram 2000; Darmency 2009; OECD 2001). However, the presence of the bolting genes and environmental triggers such as day-length, temperature or the amount of cold during the winter may induce flowering during the first year (Boudry 2002; Büttner 2010; Van Dijk 1997). Since the root crop is usually harvested during the first year while still in the vegetative phase, flowers rarely develop in sugar beet production fields. Sugar beet pollen is mostly carried by wind with insects playing a lesser role (OECD 2001). Exact percentages of outcrossing vary considerably at different locations based on numerous environmental conditions. Wind, however, is considered to be the primary vector for pollination.

B. Development of H7-1 herbicide 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-agriculture sites (Cerdeira 2006).

The management of weeds in conventional 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 requires application of the pre-emergent herbicides on the entire field because farmers cannot predict where, on their fields, weeds will emerge. With H7-1, growers have the option of applying herbicide after weeds have germinated and only in the areas of the field where there are weeds.

These 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 over two decades for introducing various genes directly into plant genomes.

[2] The applicant has described H7-1sugar 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.

APHIS authorized the first field testing of these sugar beets from 1998 through 2002. Event H7-1 sugar beets have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. The field tests were conducted in agricultural settings under physical and reproductive confinement conditions.

C. Description of the modification

Sugar beet H7-1 was produced by transformation using disarmed *Agrobacterium tumefaciens.* Sugar beet line 3S0057 (KWS SAAT AG proprietary line) cotyledons were infected with *A. tumefaciens* strain CP4 containing plasmid PV-BVGT08 (Monsanto and KWS SAAT AG 2004, pp. 20-21). This technique using disarmed *Agrobacterium* followed by selection (Howard 1990) has a history of use and has been used for transformation of a variety of plant tissues for over 20 years. 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 (Monsanto and KWS SAAT AG 2004, p. 23). Data supplied in the petition (Monsanto and KWS SAAT AG 2004, pp. 29-47) support the conclusion that event H7-1 contains the following sequences:

<u>5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)</u>

- The 35S gene promoter from a figwort mosaic virus (Gowda 1989; Sanger 1990) 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 cp4 epsps* coding region (Timko 1988) targets the protein to the chloroplast.

- The *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 (Padgette 1995) has been sequenced and encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids (Padgette 1996).

- 3’ untranslated region (transcriptional terminator and polyadenylation 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 *cp4 epsps* gene and its associated regulatory sequences over several breeding generations (Monsanto and KWS SAAT AG 2004). Statistical analyses show that glyphosate tolerance is inherited as a dominant trait in a typical Mendelian manner.

D. Potential for H7-1 to have altered disease and pest susceptibilities

APHIS assessed whether H7-1is likely to have significantly altered disease and pest susceptibility. This assessment encompassed a thorough consideration of the introduced trait and disease and pest susceptibility data from H7-1field trials.

The *Agrobacterium* transformed plants used in the generation of H7-1 were treated with an antibiotic to kill the *Agrobacterium* cells (Monsanto and KWS SAAT AG 2004, p. 19). Furthermore, DNA sequences derived from plant pests that were incorporated into 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.

Sugar beets are susceptible to a wide variety of insect pests and plant diseases (Cattanach 1991). 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 *cp4 epsps* gene and its associated regulatory sequences. H7-1 was field tested in the U.S. over five years (1998-2002) at ninety-eight field trial sites representing a wide range of environmental conditions (Monsanto and KWS SAAT AG 2004, p. 62). Plant disease was noted in thirty-six of the ninety-eight trial sites (Monsanto and KWS SAAT AG 2004, pp. 63-65). 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 between H71 and conventional sugar beet varieties in all but six field trial sites. Observations found that H7-1 exhibited 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 difference was identified at eleven sites. These differing levels of resistance and susceptibly observed between H7-1 and comparator sugar beet plants is likely due to the non-GE part of the genetic background in the H7-1 plants, because there were no trends in disease susceptibility with event H7-1 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 (Monsanto and KWS SAAT AG 2004, pp. 69-70).

Nursery trials tested the performance of different sugar beet plant varieties including H7-1 when challenged (artificial or natural infection) with plant pathogens (Monsanto and KWS SAAT AG 2004). 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 and *Rhizoctonia* root rot). H7-1 infection levels 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 and/or laboratory experiments (Estok 1989; Wan 1998). Subsequent field studies did not show increased incidence to these root 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. incognita, M. javanica* and *M. hapla*)) were monitored during the U.S. field trials (Dewar 2006, Monsanto and KWS SAAT AG 2004, pp. 66-68). No significant differences between H7-1 and conventional sugar beets 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://www.agls.uidaho.edu/ebi/vdie// and Whitney 1986), a caulimovirus and from which the promoter for the *cp4 epsps* gene originates) so silencing of the *cp4 epsps* gene should not occur.

Given the interactions between the environment, the genetic background off the cultivars used and some inherent genetic variability within sugar beet varieties, APHIS concludes that these results do not indicate an increased pest risk. Expression of CP4 EPSPS in event H7-1 sugar beet is not expected to cause plant disease or influence susceptibility of H7-1 or its progeny to disease or other pests.

E. Potential for effect on non-target organisms, including those beneficial to agriculture

Based on the data provided by the applicant and existing literature, APHIS evaluated the potential for plant pest-related impacts from event H7-1 on non-target or beneficial organisms. The inserted genetic material is not toxic and does not produce any substance that would be considered toxic (USEPA 1993). The lack of known toxicity for this enzyme suggests no potential for deleterious effects on beneficial organisms such as bees and earthworms. The high specificity of the enzyme for its substrates makes it unlikely that the introduced enzyme would metabolize endogenous substrates to produce compounds toxic to beneficial organisms. Field observations of H7-1 (Monsanto and KWS SAATAG 2004, pp. 53-70) revealed no negative effects on non-target organisms, suggesting that the production of the CP4 EPSPS protein in the plant tissues is not toxic to organisms.

Even though the likelihood of toxicity is low for the CP4 EPSPS protein, a number of researchers have conducted laboratory investigations with different types of arthropods exposed to genetically engineered crops containing the CP4 EPSPS protein (Goldstein 2003; Harvey 2003; Jamornman 2003). Representative pollinators, soil organisms, beneficial arthropods and pest species were exposed to tissues (pollen, seed, and foliage) from GE crops that contain the CP4 EPSPS protein. These studies, although varying in design, all reported a lack of toxicity observed in various species exposed to these crops (Dunfield 2003; USEPA 1993; Siciliano 1999).

APHIS has found no evidence or reason to believe that the presence of the *cp4 epsps* gene or the expressed EPSPS protein in H7-1 would have any adverse impacts on other organisms, including those beneficial to agriculture (such as earthworms, honeybees, predators or parasites of sugar

beet pests). Base on the above information, APHIS has concluded that adverse impactions to non-target organisms exposed to H7-1 are unlikely.

F. Potential for enhanced weediness or invasiveness

In the U.S., *Beta vulgaris* L. spp. *vulgaris*, is not listed as a weed by the Weed Science Society of America (2010) nor is it listed as a noxious weed species by the U.S. Federal government (7 CFR part 360; http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/). In order to assess the potential for H7-1 to become invasive and/or a weed, APHIS looked to whether H7-1's reproductive characteristics and survival biology were any different from the characteristics of conventional sugar beet varieties. One measure of a plant's potential to be invasive is the potential for the plant to produce "volunteers." Sugar beets possess few of the characteristics of plants that are notable of successful weeds (Baker 1965; Keeler 1989). As part of a bilateral agreement between the United States and Canada, USDA/APHIS and the Canadian Food Inspection Agency (CFIA) have generated documents that outline basic data requirements for developers of genetically engineered plants. One of these documents, Appendix II, outlines the environmental characterization data requirements for unconfined releases. These data and the data required in a petition for non-regulated status are designed to inform APHIS of any differences between the characteristics, including growth and seed characteristics that influence reproductive and survival biology of the transgenic plant compared to its non-transgenic counterpart.

APHIS assessed whether H7-1 is any more likely to become a weed than the isogenic non-transgenic sugar beet line. The assessment encompasses a thorough consideration of the basic biology of sugar beet and an evaluation of the unique characteristics of H7-1 under field conditions. Monsanto conducted field trials of H7-1 between 1998 and 2002 at 98 field trial sites in the U.S. (Monsanto and KWS SAAT AG 2004, p. 62) and Europe (Monsanto and KWS SAAT AG 2004, p. 70). APHIS considered data relating to plant vigor, bolting, seedling emergence, seed germination, seed dormancy and other characteristics that might relate to increased weediness (Monsanto and KWS SAAT AG 2004, pp. 70-77). Field trial data indicated that H7-1 does not exhibit characteristics that would cause it to be weedier than the parental sugar beet line. 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.

These results on growth characteristics, seed production and germination indicate that H7-1 is not different from its comparators in these respects. There is no indication that H7-1 possesses a selective advantage that would result in increased weediness or invasiveness. H7-1 lacks the ability to persist as a troublesome weed, and there would be no significant impact on current weed management practices for sugar beet cultivation, because alternative methods exist that can be used to kill weeds in the absence of using glyphosate (OECD 2001).

G. Potential of H7-1 to impact the weediness of other plants with which it can interbreed

APHIS evaluated the potential for gene introgression to occur from H7-1 to sexually compatible wild relatives and considered whether such introgression would result in weediness in those resulting hybrids. 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; Panella 2007).

Sugar beet is strongly self-incompatible (particularly tetraploid plants) although self-fertilizing plants exist in nearly every population (OECD 2001). Outcrossing is very important in developing hybrid seed every year for commercial production. The use of cytoplasmic male sterile lines is used extensively for seed production.

Distribution of Beta species in the United States

Beet species that have been introduced into the United States include *B. procumbens* Chr. Sm., *B. vulgaris* ssp. *vulgaris*, *B. vulgaris* ssp. *maritima* and *B. macrocarpa* Guss (USDA ARS 2011). The distribution of *B. procumbens* is limited to the state of Pennsylvania (USDA ARS 2011).

The only location where feral beets are recorded as having a genuine presence in the environment is in California. Wild beets are found from the San Francisco Bay area to the Mexican border (Bartsch 1999) (Figures 1 and 2). The California wild beets belong to two different taxa, *B. vulgaris* and *B. macrocarpa*, and have at least three different origins (Bartsch 2002). They evolved from escaped cultivated Swiss chard or red beet, from *B. macrocarpa*, or from hybridization of *B. vulgaris* with introduced *B. macrocarpa.* Research by Bartsch and Ellstrand (1999) identified that many populations of wild beets in California are feral varieties of Swiss chard and table beets (Bartsch 2003).

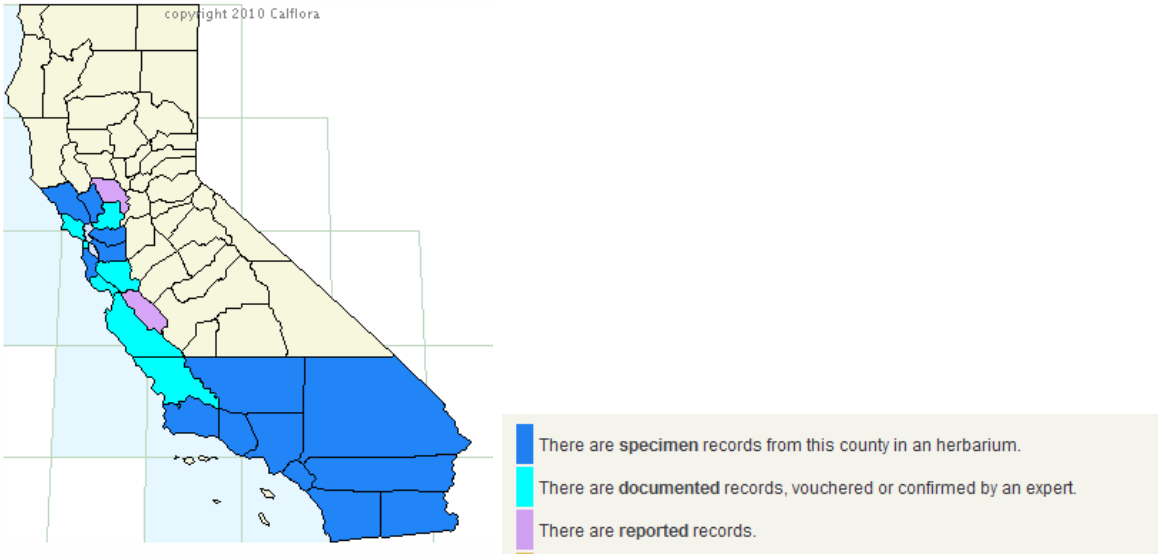

Figure 1. Distribution of *B. vulgaris* (Calflora 2011)

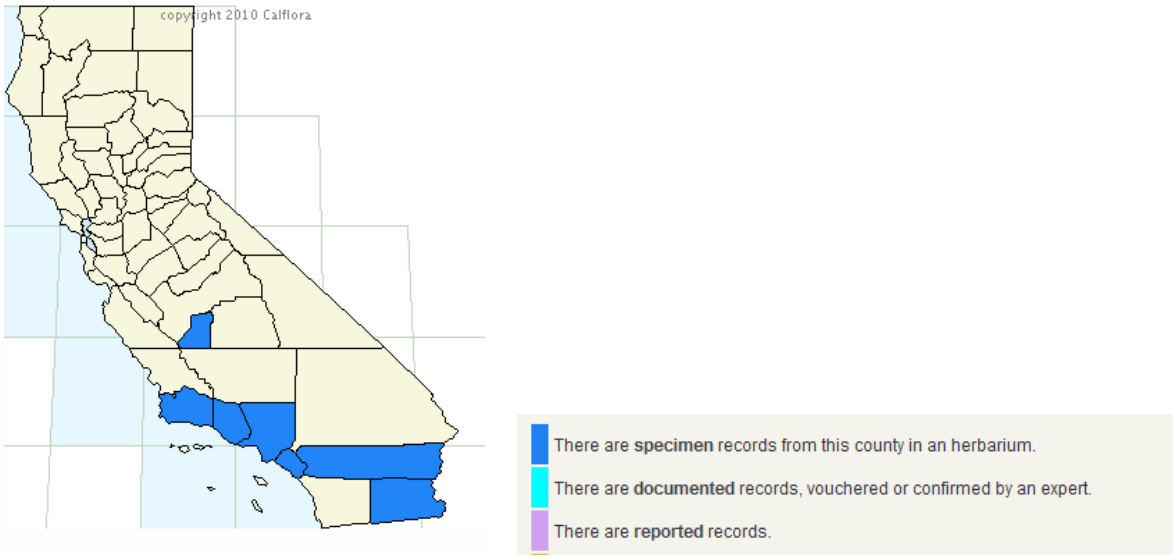

Figure 2. Distribution of *B. macrocarpa* (Calflora 2011)

Potential for H7-1 to hybridize with Beta species that occur in the U.S.

Hybrids between sugar beet and *B. procumbens* normally die at the seedling stage (Savitsky 1975; OECD 2001). Therefore, hybridization of H7-1 with the wild *B. procumbens* in Pennsylvania is unlikely to occur due to species incompatibility.

Another *Beta* species, *Beta macrocarpa,* grows in the Imperial Valley of California (Figure 2) where sugar beet root production occurs. Hybrids between sugar beet and *B. macrocarpa* are also not likely to occur. This is because *B. macrocarpa* show a high degree of self-fertilization and are known to occupy the same or overlapping geographic areas without interbreeding (Lange 1999). Also, *B. macrocarpa* usually flowers earlier than sugar beet. Bartsch speculated that *B. macrocarpa* can cross with sugar beet bolters when flowering times overlap (Bartsch 2002) based on evidence of introgression from isozyme analysis. Lewellen, however, found that *B. macrocarpa* x *B. vulgaris* when crossed in the greenhouse under ideal conditions were usually unsuccessful and the progeny were genetically unstable, further supporting the conclusion that hybrids between sugar beet and *B. macrocarpa* are unlikely (Lewellen 2003).

Where cultivated sugar beets are grown in close proximity to wild *B. vulgaris*, they can freely hybridize when flowering times overlap (Boudry 1993). In California, feral populations of *Beta vulgaris,* thought to be escaped table beet or Swiss chard, have established along the coast and pose a minor weed problem (Bartsch 1999; Johnson 1958; McFarlane 1975). These plants are not serious weed problems except when they are present in table beet, Swiss chard, and sugar beet fields (Johnson 1958; Panella 2003). Confirmed populations of *Beta vulgaris* are found in the San Francisco Bay area and along the coast where sugar beets are no longer cultivated. Wild *B. vulgaris* would be capable of crossing with flowering sugar beet plants. These beets are easily controlled with herbicides or mechanical cultivation in non- *Beta* crops. Control of weed beet in *Beta* crop fields is more difficult because young plants resemble *Beta* crops making it difficult to

remove them by mechanical means and they are tolerant of the herbicides used for weed control in beet crops.

Escape of the engineered trait into weed beet populations is possible if grown in close proximity to weedy or feral beets. APHIS concludes that the potential of the glyphosate tolerance trait moving from H7-1 to other sexually compatible *Beta* species in the United States is low, because of lack of ability to hybridize or proximity to sexually compatible plants. If the trait did move into wild species, these could not be controlled with glyphosate in H7-1 sugar beets. As glyphosate is not used for post-emergent control of wild beets in other *Beta* crops and other herbicides are effective to control wild beets in non-*Beta* crops, the potential for glyphosate tolerant weed beets to cause problems in H7-1 sugar beet fields or other crops that are resistant to glyphosate would be limited. 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).

H. Potential changes to agricultural or cultivation practices

In addition to field studies on agronomic parameters, Monsanto and KWS SAAT AG (2004, pp. 77-84) analyzed sugar beets for compositional changes as part of their submission to FDA in the consultation process. 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, however, none of the values for seed composition characteristics were outside the range of natural variability of conventional sugar beet. Therefore, the composition of H7-1 is not biologically different than conventional sugar beets.

Other than the use of glyphosate to control weeds, none of the management practices currently employed for conventional sugar beet cultivation is expected to change if H7-1 is determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. Monsanto and KWS SAAT AG (2004) studies demonstrate that the cultivation practices needed for growing H7-1 sugar beet are essentially indistinguishable from practices used to grow conventional sugar beet varieties with the exception of the glyphosate-based weed control regime. No differences in insect or disease damage were observed in field trials with H7-1. H7-1 is comparable to currently available sugar beet varieties in terms of resistance to insects and disease (Monsanto and KWS SAAT 2004, pp. 53 – 70). Because agricultural and cultivation practices would not be significantly different than that of conventional sugar beets, APHIS does not foresee changes in on insects or diseases damage or control measures employed due to agricultural or cultivation practices with H7-1.

I. Potential impacts from transfer of genetic information to organisms with which H7-1 cannot interbreed

APHIS examined the potential for the new genetic material inserted into H7-1 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. Horizontal gene transfer and expression of DNA from a plant species to other species is highly unlikely to occur based on the following reasons.

The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields of science. Horizontal gene transfer and expression of DNA from a plant species to bacteria or animal species is unlikely to occur (Keese 2008).

1. Many genomes (or parts thereof) from bacteria 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. Therefore the likelihood of any impact or new horizontal gene transfer that is not already capable of taking place in the soil is extremely unlikely.
2. No evidence has been identified for any mechanism by which sugar beet genes could be transferred to humans or animals, or any evidence that such gene transfer has occurred for any plant species during evolutionary history, despite animals and humans eating large quantities of plant DNA. 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).
3. Transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced.

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 extremely unlikely (FDA 1998). Therefore APHIS concludes that horizontal gene transfer is highly unlikely to occur and thus poses no significant plant pest risk.

J. Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if event H7-1 sugar beet is unlikely to pose a plant pest risk under full deregulation. Based on the information provided by the applicant and the lack of atypical responses to disease or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, weedy characteristics of H7-1 or other plants with which it can interbreed, changes to agricultural or cultivation practices, and the unlikelihood of horizontal gene transfer, APHIS has concluded that event H7-1 sugar beet is highly unlikely to pose a plant pest risk.

K. References

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