MONSANTO

Petition for Determination of Nonregulated Status for Roundup Ready® Sugar Beet Event H7-1

The undersigned submit this request under 7 CFR Part 340.6 to request that the Administrator make a determination of nonregulated status that the article should not be regulated under 7 CFR Part 340.

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Summary

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act of 2000 (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. The APHIS regulations at 7 CFR § 340.6 provide that an applicant may petition APHIS to evaluate submitted data and information to determine that a particular regulated article does not present a plant pest risk and should therefore not be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition may be granted, thereby allowing unrestricted introduction of the article in the U.S.

Monsanto Company and KWS SAAT AG (hereafter referred to as Monsanto and KWS, respectively) submit this request to APHIS for a determination of nonregulated status for Roundup Ready® sugar beet event H7-1, also known simply as event H7-1, and all progeny derived by conventional plant breeding from this event. The glyphosate tolerance of event H7-1 was imparted by the insertion of a *cp4 epsps* gene cassette that encodes the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein from *Agrobacterium* sp. strain CP4. Transformed plants treated with glyphosate, the active ingredient in Roundup® agricultural herbicides, are unaffected because the continued action of the expressed tolerant EPSPS enzyme provides the plant's need for aromatic amino acids (OECD, 1999; Padgette et al., 1996).

Sugar beet, *Beta vulgaris* ssp. *vulgaris,* has a long history of safe agricultural production and use. The objective of the genetic modification in event H7-1 was to simplify and improve weed management practices in sugar beet by the addition of the CP4 EPSPS protein to confer tolerance to glyphosate. The donor organism, *Agrobacterium* sp. strain CP4, was the source of the *cp4 epsps* gene coding sequence to impart glyphosate tolerance. The *cp4 epsps* gene coding sequence is well characterized and the CP4 EPSPS protein product is homologous to plant and microbial EPSPS proteins that are widely prevalent and have a long history of safe use.

The transformation vector, PV-BVGT08, containing the *cp4 epsps* coding and regulatory sequences, was introduced into sugar beet by an *Agrobacterium tumefaciens* plant transformation system to produce event H7-1. Molecular analyses of event H7-1 were performed to characterize the single stable site of insertion into the plant genome. Southern blot analyses confirmed that event H7-1 contains one copy of the transformation cassette inserted at a single locus in the plant genome. No additional elements from the transformation vector, linked or unlinked to the intact gene cassette, were detected in the sugar beet genome. Event H7-1 does not contain any detectable plasmid backbone sequence. These data support the conclusion that only the single protein of interest, CP4 EPSPS, is encoded by the inserted DNA in event H7-1.

Segregation analysis of the glyphosate-tolerant phenotype across four generations confirmed that glyphosate tolerance is inherited as a single Mendelian trait and confirmed

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the stability of the *cp4 epsps* gene. Southern blot analysis of DNA extracted from plants across three generations further confirmed the stability of the inserted gene in event H7-1. The mean levels of the CP4 EPSPS protein, on a fresh weight basis, were estimated to be 161 μg/g and 181 μg/g in event H7-1 top and root tissues, respectively.

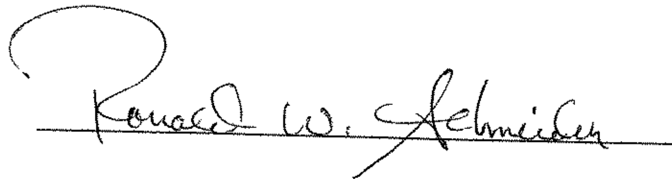
Phenotypic data and information demonstrate that the regulated article, event H7-1, is no more likely to pose a plant pest risk than conventional sugar beet, based on the following: (1) pest susceptibility observations confirmed that event H7-1 is no more susceptible to diseases or insect pests than conventional sugar beet; (2) agronomic characteristics, performance and morphological data have demonstrated that event H7-1 is not meaningfully different morphologically and agronomically than conventional sugar beet, indicating there is no competitiveness or weediness difference between event H7-1 and conventional sugar beet; (3) compositional and quality component analyses have shown that event H7-1 is compositionally equivalent to its control for key compositional constituents and the antinutrient saponin; and (4) the only phenotypic difference observed between event H7-1 and conventional sugar beet is the tolerance to glyphosate conferred by the CP4 EPSPS protein. As such, the *cp4 epsps* gene, including the regulatory sequences, and the produced CP4 EPSPS protein do not confer plant pest characteristics to event H7-1.

The environmental consequences of the introduction of event H7-1 have been considered and there is no reason to believe that event H7-1 would exhibit a significant potential to harm organisms beneficial to plants or to nontarget organisms, including threatened or endangered species, based on the following: (1) the agronomic consequences of volunteer sugar beet would be minimal because the plants are easily controlled by currently used agronomic practices; (2) there is no evidence of altered ecological interactions compared to conventional sugar beet; (3) the EPSPS family of proteins, and specifically CP4 EPSPS as produced in a number of Roundup Ready crops including corn, soybean, canola, cotton and sugar beet, has been shown to be comparable to the EPSPS proteins present in other food crops and common microbes; and (4) there has been no reported adverse environmental impact from the commercial planting of other Roundup Ready crops (e.g., soybean, corn, cotton and canola) which contain the CP4 EPSPS protein.

In conclusion, the data and information in this request demonstrate that Roundup Ready sugar beet event H7-1 is unlikely to pose a greater plant pesk risk than the conventional sugar beet variety from which it was derived. Thus, these data and information provide the factual grounds why event H7-1 should not be regulated under 7 CFR § 340. Therefore, Monsanto and KWS request a determination of nonregulated status from APHIS, such that Roundup Ready sugar beet event H7-1, and any progenies derived from crosses between this event and other sugar beet varieties, are no longer considered regulated articles.

Certification

The undersigned certify that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioners which are unfavorable to the petition.



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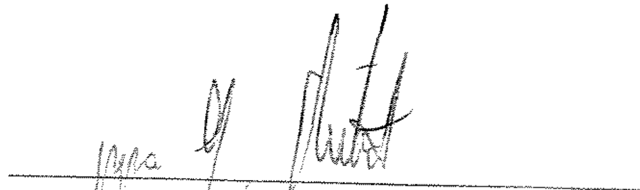

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Abbreviations, Definitions and Acronyms[1]

o ....Degrees
~ ....Approximately
3' ....The distal, or growing end, of an mRNA transcript; the end nearest to or containing the polyadenylation region in the T-DNA sequence
5' ....The proximal, or start end, of an mRNA transcript; the end nearest to the promoter in the T-DNA sequence
A............................Adenine
aad........................Gene encoding spectinomycin and streptomycin resistance
aPAD....................acute Population Adjusted Dose
bp...........................Nucleotide base pairs
C ....Cytosine or Centigrade
CFIA.....................Canadian Food Inspection Agency
CMS ....Cytoplasmic male sterility
CP4 EPSPS ....EPSPS protein encoded by the *cp4 epsps* gene
cp4 epsps.............Gene derived from *Agrobacterium* sp. strain CP4, encoding the CP4 EPSPS protein
CTAB...................Cetyltrimethylammonium bromide
CTP ....Chloroplast Transit Peptide
*ctp*2.......................DNA sequence coding for CTP variant 2
DNA.....................Deoxyribonucleic acid
dATP....................Deoxyadenosine triphosphate
dCTP ....Deoxycytidine triphosphate
E9 3'.....................3' polyadenylation region of the pea *rbc*S E9 gene
E. coli...................*Escherichia coli*
EDTA...................Ethylenediaminetetraacetic acid
ELISA ....Enzyme-linked immunosorbent assay
EPSP 5-Enolpyruvylshikimate-3-phosphate
EPSPS ....5-Enolpyruvylshikimate-3-phosphate synthase
FMV ....Figwort mosaic virus
G............................Guanine
g.............................gram
gox....................... Glyphosate oxidoreductase
H7-1 ....Event designation for glyphosate-tolerant, transformed sugar beet
HCl ....Hydrochloric acid
HRP......................Horseradish peroxidase
kb..........................Nucleotide kilobase pairs
kD.........................kiloDalton
L ....Linné
LB.........................Left Border
M ....Molar
ml ....milliliter

[1] Standard abbreviations, e.g., units of measure, are used according to the format described in 'Instructions to Authors' in the *Journal of Biological Chemistry*.

mM.......................millimolar
MO.......................Monogerm
Monocarp ....one fruit (true seed) per node of inflorescence
Multicarp ....several fruits (true seed) per node of inflorescence
mRNA..................Messenger RNA
MW......................Molecular weight
NaOH...................Sodium hydroxide
Na_2HPO_4..............Sodium phosphate dibasic
OD........................Optical density
OECD..................Organization for Economic Cooperation and Development
ori.........................Origin of replication
ori-V.....................Bacterial origin of replication from RK2 plasmid
ori-322.................Bacterial origin of replication from *E. coli* plasmid pBR322
PCR......................Polymerase chain reaction
pBR322................Plasmid cloning vector of bacterial origin
PEP.......................Phosphoenolpyruvate
P-FMV ....35S promoter from figwort mosaic virus
PV-BVGT08........*Agrobacterium* plasmid vector used to transform event H7-1
RB........................Right Border
rop........................A segment of plasmid pBR322 critical to providing maintenance and copy number control of plasmids in bacterial hosts, such as *E. coli*
rpm.......................Revolutions per minute
RR.........................Genotype that is homozygous for glyphosate tolerance
Rr..........................Genotype that is heterozygous for glyphosate tolerance
rr..........................Genotype that is homozygous for glyphosate sensitivity
RT.........................Room temperature
S3P.......................Shikimate-3-phosphate
SDS......................Sodium dodecyl sulfate
sp..........................Species
ssp........................Subspecies
SSC......................Saline-sodium citrate buffer. 20X SSC is 3 M sodium chloride, 0.3 M sodium citrate
T...........................Thymine
T-DNA.................Transferred DNA
TE buffer.............Tris-EDTA buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
TKW ....Thousand kernel weight
TMB.....................3,3',5,5' Tetramethylbenzidene, peroxidase substrate
Tn7.......................Bacterial transposon that carries the *aad* gene for resistance to spectinomycin and streptomycin
Tris.......................Tris (hydroxymethyl)aminomethane
*uid*A......................Gene that codes for the β-D-glucuronidase (GUS) protein
UF........................Uncertainty factor
U.S.C...................United States Code
UV........................Ultraviolet
var........................Variety

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I. RATIONALE FOR REQUEST FOR A DETERMINATION OF NONREGULATED STATUS

A. Basis for Request for a Determination of Nonregulated Status Under 7 CFR Part 340.6

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has the responsibility, under the Plant Protection Act of 2000 (7 U.S.C. § 7701-7772), to prevent the introduction or dissemination of plant pests into the U.S. Under this authority, APHIS has published regulations at 7 CFR 340 entitled "Petition for Determination of Nonregulated Status", which provide that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not represent an increased plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition may be granted, thereby allowing unrestricted introduction of the article in the U.S.

B. Roundup Ready Sugar Beet Event H7-1

Roundup Ready sugar beet event H7-1, assigned the OECD unique identifier KM-ØØØH71-4, has been genetically modified to tolerate application of Roundup agricultural herbicides in order to simplify and improve weed management practices in sugar beet production. Weed management is an expensive, labor intensive, and, in some cases, complicated operation necessary for optimal production efficiency and yield of sugar beets. No other single currently approved herbicidal active ingredient offers the broad spectrum weed control and application flexibility afforded by Roundup agricultural herbicides. Instead, farmers must resort to using several applications of multiple herbicides with highly variable costs and performance efficiencies, in addition to the frequent utilization of hand labor.

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry Number 1071-83-6), the active ingredient in the nonselective, foliar-applied, broad-spectrum, post-emergent herbicides within the Roundup agricultural herbicide family (Baird et al., 1971; Malik et al., 1989), is the world's most widely used herbicide. This is primarily because of its excellent weed control capabilities and its favorable environmental and safety characteristics (Geisy et al., 2000; Williams et al., 2000). However, the sensitivity of crop plants to glyphosate has prevented the in-season use of this herbicide over the top of conventional crops. The tools of biotechnology enabled the development of crops that are tolerant to this herbicide (Barry et al., 1992; Padgette et al., 1996). The extension of the use of Roundup agricultural herbicides to allow in-crop application in crops such as sugar beet provides a simple and effective weed control option for growers.

Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaf weeds. Glyphosate has excellent environmental features, such as rapid soil binding (resistance to leaching) and biodegradation (which decreases persistence), as well as extremely low toxicity to mammals, birds and fish (Malik et al., 1989; Geisy et al.,

2000; Williams et al., 2000). Glyphosate is classified by the EPA as Category E (evidence of non-carcinogenicity for humans) (57 FR 8739). The use of event H7-1 for sugar beet production would enable farmers to use Roundup agricultural herbicides for effective control of weed pests while receiving the benefits of its favorable environmental and safety characteristics. Roundup Ready sugar beets can positively impact current agronomic practices by:

- Offering growers a new, broad-spectrum weed control option,
- Increasing flexibility to treat weeds on an "as needed" basis,
- Offering less dependence on use of preemergent herbicides,
- Increasing grower flexibility by reducing crop rotation restrictions associated with several pre- and post-emergent herbicides currently on the market,
- Offering growers a novel mode of action as an alternate herbicide in a weed-resistance management program in-season, and
- Allowing the use of a herbicide with favorable environmental characteristics.

C. Submissions to Other Regulatory Agencies

C.1. Submission to FDA

Event H7-1 is within the scope of the FDA policy statement concerning regulation of products derived from new plant varieties as published in the Federal Register on May 29, 1992. In compliance with this policy, Monsanto consulted FDA and in April, 2003, submitted to FDA a food and feed safety and nutritional assessment summary for Roundup Ready sugar beet event H7-1.

C.2. Submission to EPA

The U.S. EPA has authority over the use of all pesticide substances, including herbicides, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). EPA granted the registration of the Roundup Ultra® herbicide label (EPA Reg. No. 524-475) for use over the top of Roundup Ready sugar beet on March 31, 1999, with supporting glyphosate residue tolerances established on April 14, 1999 (64 FR 18360). Based on a memorandum of understanding between U.S. EPA and Monsanto, it was concluded that data already generated on Roundup Ready sugar beet adequately demonstrated the residue profile for other events of sugar beet which contain the same gene, and that further studies would not provide any new information. Therefore, the existing glyphosate residue data in sugar beet roots, tops and dried pulp are sufficient to support the label for the application of glyphosate to future Roundup Ready sugar beet, such as event H7-1.

C.3. Submission to foreign governments

Regulatory submissions for production approval of event H7-1 were made in April, 2003 to Health Canada for novel food approval and to the Canadian Food Inspection Agency (CFIA) for environmental release and novel feed approval. Regulatory submissions will

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be made to countries with established regulatory systems that import U.S. sugar beet processed products to gain food and feed approvals. Food approval for importation of food products into Japan was received from the Japanese Ministry of Health, Labor and Welfare (MHLW) in June, 2003, and a feed importation submission has been made and is presently under review by the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF). A submission to the European Union (EU) under Directive 90/220 EEC has been made, which has been upgraded to comply with Directive 2001/18/EC, and a submission following guidance under the forthcoming EU Genetically Modified Food and Feed Regulation is planned.

II. THE SUGAR BEET FAMILY

A number of excellent references provide a breadth of information on sugar beet, including an OECD Consensus document (OECD, 2001) and a similar document developed by the CFIA (CFIA, 2002). In accordance with Section 99-3 of the USDA's Guide for Preparing and Submitting a Petition for Genetically Engineered Plants, the OECD Consensus document is cited as a broad review of the sugar beet family. Also pertinent to this petition is an OECD Consensus document on glyphosate, the active ingredient in the Roundup family of agricultural herbicides (OECD, 1999). More detailed information on sugar beet processing technology and quality parameters is provided in Beet – Sugar Technology (McGinnis, 1982). These references were used to provide the information below.

A. Scientific Name and Taxonomic Classification of Sugar Beet

Beta vulgaris L. ssp. *vulgaris* var. *altissima* is the scientific name for sugar beet. Sugar beet belongs to the family *Chenopodiaceae* (OECD, 2001). This family includes approximately 1400 species divided into 105 genera (CFIA, 2002). The genus *B. vulgaris* comprises several cultivated forms of *B. vulgaris* ssp. *vulgaris*. The genus *Beta* is sub-divided into four sections: *Beta*, *Corollinae*, *Nanae* and *Procumbentes* (OECD, 2001).

B. Growth and Reproduction Characteristics of Sugar Beet

B. vulgaris is normally a biennial species; however, under certain environmental conditions early in the growing season that induce vernalization it can act as an annual. The sugar beet plant develops a large succulent taproot in the first year and a seed stalk the second year. Typically sugar beet root crops are planted in the spring and harvested in the autumn of the same year. For seed production, however, an overwintering period with cold temperatures of 4-7°C (vernalization) is required for the root to bolt in the next growing season and for the reproductive stage to be initiated (CFIA, 2002).

During the first growing season, the vegetative stage, the sugar beet plant is described as having glabrous leaves, forming a rosette from an underground stem, that are ovate to cordate in shape and dark green in color. A white fleshy taproot develops, prominently swollen at the junction of the stem. During the second growing season, the reproductive stage, a flowering stalk elongates from the root, a process commonly referred to as bolting. This angular seed stalk forms an inflorescence and grows approximately 1.2 to 1.8 meters tall. A large petiole leaf develops at the base of the stem with small leaves, while further up the stem there are less petiole leaves and finally sessile leaves. At the leaf axis, secondary shoots develop, forming a series of indeterminate racemes. Sugar beet produces a perfect flower consisting of a tricapelate pistil surrounded by five stamens and a perianth of five narrow sepals. These flowers are small and sessile, occurring singly or in clusters. Petals are absent and each flower is subtended by a slender green bract (CFIA, 2002).

As discussed previously, vernalization temperatures are needed for initiation of the reproductive phase, with most cultivars of sugar beet requiring 90 to 110 days of exposure to the inductive temperatures for reproductive development. Flower formation commences on the top shoot and flowers mature from the base upwards, with secondary shoots following afterwards. The sugar beet plant flowers for a duration of about four weeks. Flowers open mostly in the morning, but continue throughout the day, with the stigmas remaining receptive for more than two weeks (OECD, 2001).

During flowering, the pollen grains produced are round and have numerous indentations. The number of pollen grains per anther is estimated at approximately 17,000, with the pollen viability limited to a maximum of 24 hours, depending on environmental conditions, especially moisture. Pollen is transported primarily by wind currents and, to a much lesser extent, by insects such as bees (OECD, 2001).

The ovary forms a fruit, which is embedded in the base of the perianth of the flower. Each fruit contains a single seed whose shape varies from round to kidney-shaped. The ovaries are enclosed by the common receptacle of the flower cluster. A monogerm seed is formed when a flower occurs singly. Multigerm beet seed is formed by an aggregation of two or more flowers (CFIA, 2002).

The genus *Beta* exists in diploid, tetraploid and hexaploid forms with a chromosome number of x=9. Most of the sugar beet varieties grown since the 1970s have been triploid hybrids. The development of hybrid sugar beet was made possible by the discovery of cytoplasmic male sterility (CMS) used in conjunction with polyploidy. Breeding programs utilizing the CMS lines to form triploid hybrids have allowed the development of superior sugar beet varieties with higher root yield and higher sugar content, better extraction yield (juice purity), higher seed germination percentages, lower tendency to "bolt", physical attributes of the root well adapted to mechanical harvesting, and higher resistance to leaf diseases (OECD, 2001).

C. History of Sugar Beet Development

Beet was a well-established vegetable in ancient Greece and Rome. The earliest literary sources of beet represented several leafy forms, commonly referred to as chards. The first known description of beet chards is by Aristotele (*circa* 350 BC), who described red chard, and Theophraastos (*circa* 300 BC) who recognized two different beets, white and black, referring to the light and dark appearance of the leaves. The use of the roots from beets are referenced both for culinary and medicinal purposes by Roman writers. While beet was established in classical times, there are no archaeological records of *Beta vulgaris* from the pre-classical times, and it is not known when the root beet was domesticated (OECD, 2001).

Beta vulgaris L. ssp. *maritima*, wild sea beet, is regarded as the mother species of the *Beta* beets, including fodder beet, sugar beet, beetroot, yellow beet and swiss chard. It is indigenous to European coastal regions, particularly the Mediterranean region. In

Europe, *B. vulgaris* species with distinctly swollen roots were cultivated in the Middle Ages. Central European types of *B. vulgaris* were presumed to have descended from those used in Arabian horticulture in Spain. These beet plants were eventually taken to the Netherlands, where they were cultivated beginning in 1500, then to the Palatinate region, later spreading to Germany as "Burgundy beet". In 1747, a pharmacist named Markgraf discovered that the sweet substance in beets was sucrose, though at a relatively low concentration of approximately 6%. In 1786, the breeder Achard selected from 23 local varieties a plant from the Halberstadt area for beet-sugar production. Subsequent breeders, Koppy and Sohn, selected the local variety "white Silesian sugar beet", with this submerged-root variety being credited as the mother type for all sugar beet varieties. Later a student of Markgraf built the first factory intended to extract sugar from the root of sugar beet in 1801, and produced the first variety, White Silesian. Further breeding efforts in the following 70 years in Europe produced beet varieties with sugar contents of about 16%, whereas the sugar content of today's varieties typically ranges from 18-20%. Sugar beet, as a crop, was introduced in North America around 1830 (OECD, 2001), and was produced on about 1.3 to 1.4 million acres in the U.S. during the 2001 and 2002 seasons, respectively (USDA-NASS, 2002).

III. DESCRIPTION OF THE TRANSFORMATION SYSTEM

A. Transformation System

Event H7-1 was developed through *Agrobacterium*-mediated transformation of a sugar beet variety used in plant breeding. *Agrobacterium*-mediated transformation is a well-documented process for the transfer and integration of exogenous DNA into a plant's nuclear chromosome (White, 1989; Howard et al., 1990).

Cotyledons derived from sterile seedlings of the diploid sugar beet line 3S0057 were used as the explant source. These cotyledons were immersed in an *Agrobacterium* suspension and co-cultured for two to four days. The explants were then transferred to selective media containing 500 mg/l carbenicillin to eliminate the agrobacteria. Glyphosate was used for selection of glyphosate-tolerant tissue, with tissue containing a genetic insertion to confer glyphosate tolerance assigned a unique number, such as event H7-1. After approximately seven weeks, the developed plantlets were transferred to rooting media and placed in a greenhouse.

B. Characteristics of the Recipient Sugar Beet Parent

The sugar beet parental material used for the transformation was a KWS proprietary multigerm line designated 3S0057. Transformation and selection were performed by KWS. The initial transformed sugar beet material, selected for tolerance to glyphosate, was designated event H7-1 and the resulting plant, as described above, was the initial breeding line, identified as 6401VH. All subsequently developed Roundup Ready sugar beet breeding lines and variety candidates, discussed within or subject to this Petition, were derived by traditional plant-breeding methods. Standard cultivation methods used in conventional sugar beet breeding were used for development of event H7-1 breeding lines and variety candidates, except for the additional benefit of allowing in-crop use of Roundup agricultural herbicides for broad spectrum weed control. The breeding origins of event H7-1 and the conventional control sugar beet plants used in the molecular characterization, field evaluations and compositional analyses are shown in Figures III-1 and III-2. An inventory of event H7-1 and its progeny used in regulatory studies is presented in Table III-1.

Figure III-1. Process flow diagram for transformation, selection, regeneration and evaluation of event H7-1

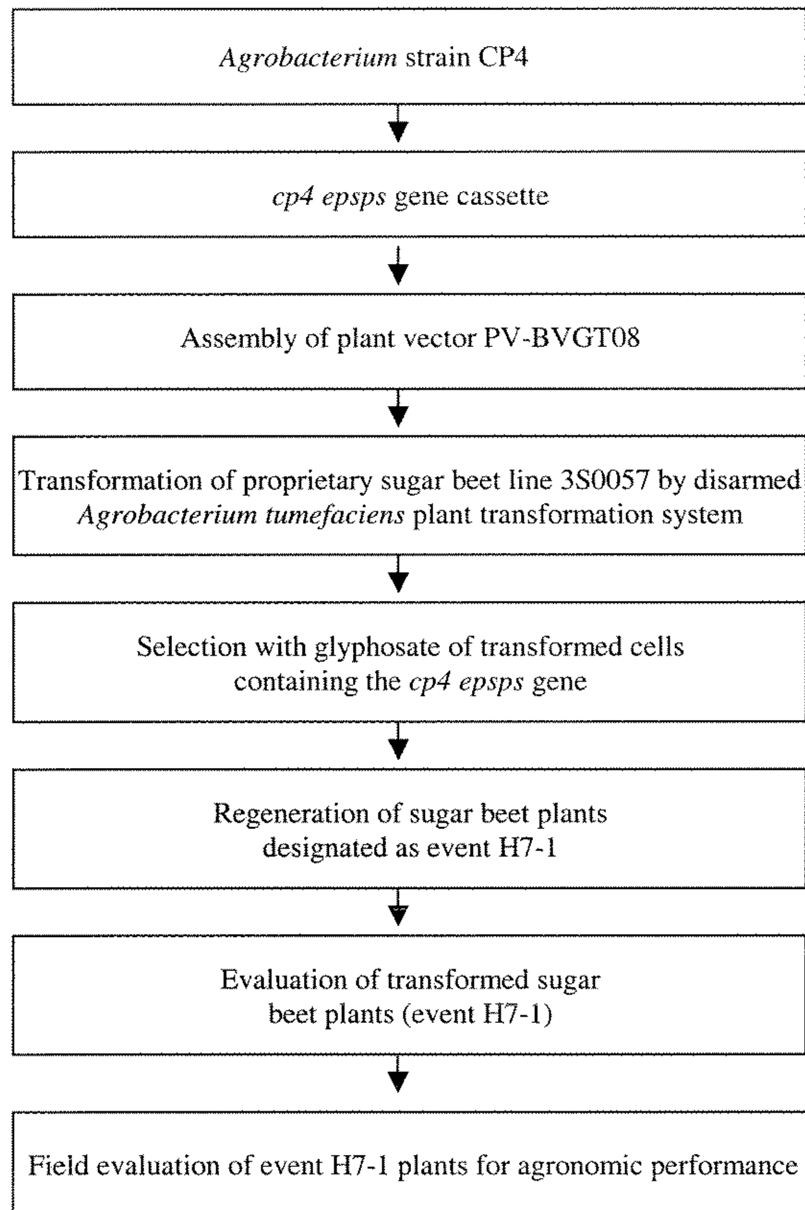
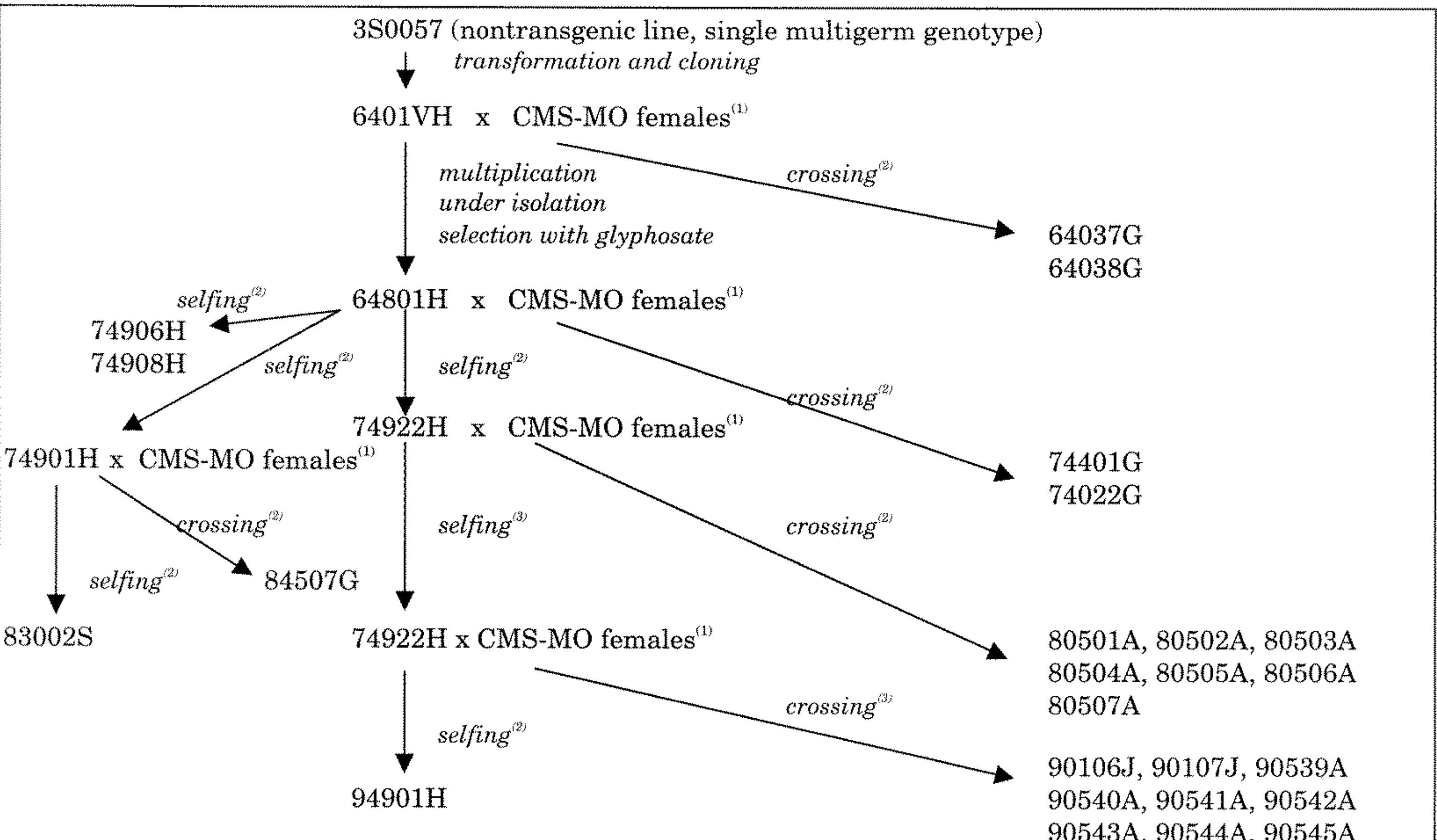


Figure III-2. Breeding history and event H7-1 progeny used in regulatory studies



Legend:
(1) Monogerm sugar beet material (MO). Cytoplasmic male sterile plants (CMS).
(2) Segregating progenies. Tolerant plants containing event H7-1 were selected by applications of glyphosate.
(3) Segregating progenies. Tolerant plants containing event H7-1 were identified using a PCR method.

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Table III-1. Inventory of event H7-1 sugar beet progeny used in regulatory studies

Event H7-1 plants (Line ID)	Studies
6401VH, 64801H, 74901H, 74922H, 83002S	Molecular characterization, segregation data on inheritance.
74022G	Compositional data (1999* field trials), protein expression data (1999*), agronomic field testing (1999*).
80503A	Compositional data (1999*), protein expression data (1999*), agronomic field testing (1999*).
80502A, 80504A, 80505A, 80506A, 80507A	Compositional data (1999*), protein expression data (1999*), segregation data on inheritance
94901H, 90106J, 90107J, 90539A, 90540A, 90541A, 90542A, 90543A, 90544A, 90545A	Segregation data on inheritance

Legend:
[*] Indicates year in which the studies or field trials were performed, which may not correspond to the year in which the seed was produced as indicated in Figure III-2.

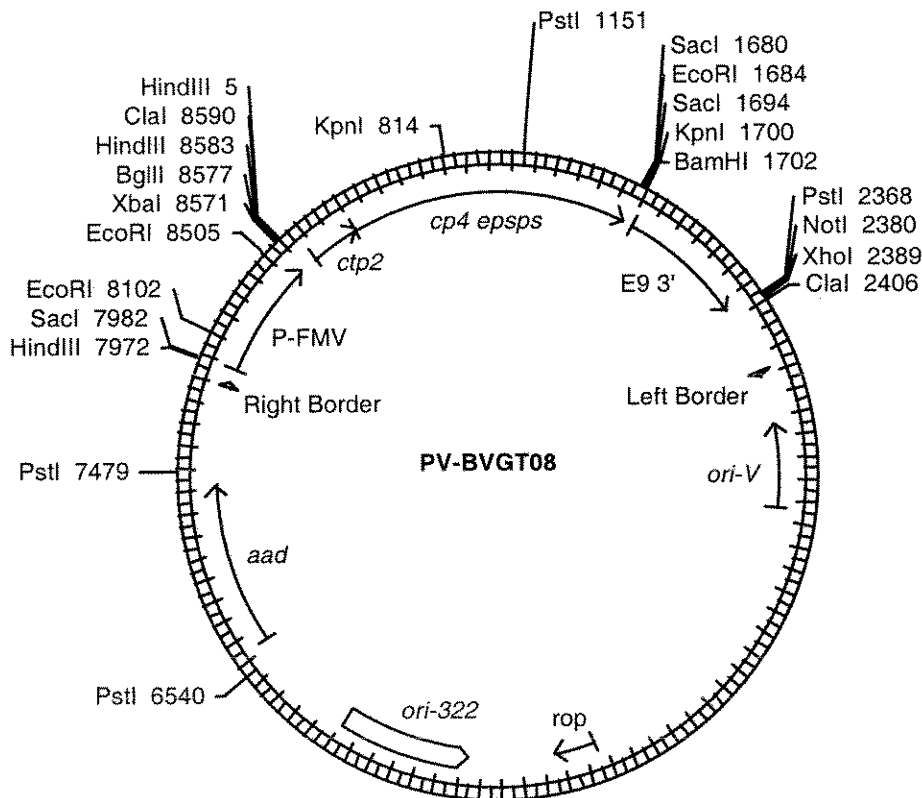
CBI-Deleted

IV. DONOR GENES AND REGULATORY SEQUENCES

A. Plasmid Vector PV-BVGT08

A disarmed, binary *Agrobacterium tumefaciens* transformation vector, designated PV-BVGT08, was used to produce event H7-1. This vector contains a region of DNA (T-DNA) that is delineated by the right and left border sequences and is made up of a single *cp4 epsps* expression cassette. The same *cp4 epsps* coding region has been used in several other Roundup Ready crops that have been previously reviewed and granted nonregulated status by the USDA, including soybean (93-258-01p), cotton (95-045-01p), corn (96-317-01p), canola (98-216-01p) and sugar beet event 77 (98-173-01p). A plasmid map of PV-BVGT08 is presented in Figure IV-1. Some restriction endonuclease sites with base pair locations are shown, including those identified in the text and used in the molecular genetic analyses.

Figure IV-1. Plasmid map of vector PV-BVGT08



Plasmid vector PV-BVGT08 contains DNA sequences that define the extent of the T-DNA that can be transferred into the plant genome. These are termed the Right Border (RB) and Left Border (LB) regions, and contain sequences necessary for transfer of T-DNA into the plant cell. In PV-BVGT08, the RB region is located 5' to the P-FMV::*ctp2*::*cp4 epsps*::E9 3' gene cassette (nucleotide positions 7601-7965 on the PV-BVGT08 map), that is, nearest to the P-FMV promoter (see Figure IV-1). In PV-BVGT08 the LB is located 3' to the P-FMV::*ctp2*::*cp4 epsps*::E9 3' gene cassette (nucleotide positions 2403-2862 on the PV-BVGT08 map), that is, nearest to the E9 3' polyadenylation signal.

The genetic elements present between the T-DNA border sequences, in order, from Right Border to Left Border are: the figwort mosaic virus 35S promoter (P-FMV), a chloroplast targeting sequence from *Arabidopsis thaliana* (*ctp2*), the EPSPS coding region from *Agrobacterium* sp. strain CP4 (*cp4 epsps*) and the E9 3' polyadenylation signal from pea (*Pisum sativum*). The promoter, targeting sequence, coding region, and polyadenylation signal comprise the P-FMV::*ctp2*::*cp4 epsps*::E9 3' gene cassette. This same gene cassette is present in other Roundup Ready crops previously reviewed and granted nonregulated status by the USDA: sugar beet event 77 (98-173-01p), cotton (95-045-01p) and canola (98-216-01p).

In addition, PV-BVGT08 contains a bacterial selectable marker gene, *aad*, that provides resistance to spectinomycin and streptomycin, as well as DNA origin of replication sequences (*ori-V* and *ori-322*) necessary for replication and maintenance of the plasmid PV-BVGT08 in bacteria. All of these genetic elements are located outside of the T-DNA, and, as expected, have not been introduced into event H7-1. A complete description of each genetic element in PV-BVGT08 is presented in Table IV-1.

B. *cp4 epsps* Gene

Roundup Ready sugar beet event H7-1 plants contain the *cp4 epsps* gene and produce the CP4 EPSPS protein, which imparts tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides. The *cp4 epsps* gene was isolated originally from *Agrobacterium* sp. strain CP4 and produces an enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which, unlike most native plant and microbial EPSPS enzymes, is naturally tolerant to glyphosate (Padgette et al., 1995). EPSPS catalyzes the formation of 5-enolpyruvylshikimate-3-phosphate (EPSP), from shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP), in both microorganisms and plants. EPSP is an intermediate required for the production of aromatic amino acids (Herrmann, 1983; Haslam, 1974).

Table IV-1. Genetic elements in plasmid vector PV-BVGT08

Genetic Element	Size (kb)	Description
Right Border	0.025	A 21-25 bp nucleotide sequence that acts as the initial point of DNA transfer into plant cells, originally isolated from *A. tumefaciens* plasmid pTiT37 (Depicker et al., 1982).
P-FMV	0.672	The 35S gene promoter from a modified figwort mosaic virus (FMV) (Sheperd et al., 1987; Richins et al., 1987; Gowda et al., 1989; Sanger et al., 1990).
ctp2	0.31	The N-terminal chloroplast transit peptide sequence from the *Arabidopsis thaliana epsps* coding region (Timko et al., 1988).
cp4 epsps	1.363	The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) coding region from *Agrobacterium sp.* strain CP4 (Padgette et al., 1995).
E9 3'	0.63	The 3' end of the *Pisum sativum rbcS* E9 gene, containing polyadenylation sites that direct mRNA processing and polyadenylation (Coruzzi et al., 1984; Morelli et al., 1985).
Left Border	0.025	A 21-25 bp nucleotide sequence that delimits the T-DNA transfer into plant cells, originally isolated from *A. tumefaciens* plasmid pTi15955, a derivative of the octopine type plasmid, pTiA6 (Barker et al., 1983).
ori-V	0.393	A vegetative origin of DNA replication, originally isolated from plasmid RK2 (Rogers et al., 1987).
ori-322	0.629	A plasmid origin of DNA replication that permits maintenance of the plasmid in bacterial hosts such as *E. coli* (Sutcliffe, 1979).
rop	0.191	A segment of plasmid pBR322 that represses the formation of RNA primer critical to maintenance of the plasmid in bacterial hosts such as *E. coli.* (Bolivar et al., 1977; Cesareni et al., 1982).
aad	0.789	The bacterial gene encoding the Tn7 AAD 3' adenyltransferase, conferring spectinomycin and streptomycin resistance (Fling et al., 1985).

The native *Agrobacterium* gene sequence was modified to create a synthetic gene that allows greater production of the native CP4 EPSPS protein in plants (Padgette et al., 1995). Bacterial genes, like those from *Agrobacterium,* have several features that reduce their ability to function efficiently in plants. Therefore, plant-preferred versions of these genes have been synthesized and used in developing the plasmid vectors (Della-Cioppa et al., 1986 and 1987; Shah et al., 1986). The CP4 EPSPS and native sugar beet EPSPS enzymes are functionally equivalent, except for their affinity to glyphosate.

The *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 has been completely sequenced and encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). The *ctp2::cp4 epsps* gene sequence, present in event H7-1, is approximately 1.7 kb in size. The deduced amino acid sequence of the CP4 EPSPS with the CTP2 transit peptide is shown in Figure IV-2.

The CP4 EPSPS used in the transformation of event H7-1 shares greater than 99.4% nucleotide sequence and greater than 99.7% amino acid sequence identity to the native

Agrobacterium sp. strain CP4 EPSPS. The amino acid sequence is predicted to be identical to that of the CP4 EPSPS protein produced in other Roundup Ready crops previously reviewed and granted nonregulated status by the USDA, including soybean (93-258-01p), cotton (95-045-01p), corn (96-317-01p) and sugar beet event 77 (98-173-01p).

Figure IV-2. Deduced amino acid sequence of the CP4 EPSPS protein in event H7-1
Sequence includes the CTP2 transit peptide (amino acids 1-76).

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  1 MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTQQHP RAYPISSSWG
 51 LKKSGMTLIG SELRPLKVMS SVSTACMLHG ASSRPATARK SSGLSGTVRI
101 PGDKSISHRS FMFGGLASGE TRITGLLEGE DVINTGKAMQ AMGARIRKEG
151 DTWIIDGVGN GGLLAPEAPL DFGNAATGCR LTMGLVGVYD FDSTFIGDAS
201 LTKRPMGRVL NPLREMGVQV KSEDGDRLPV TLRGPKTPTP ITYRVPMASA
251 QVKSAVLLAG LNTPGITTVI EPIMTRDHTE KMLQGFGANL TVETDADGVR
301 TIRLEGRGKL TGQVIDVPGD PSSTAFPLVA ALLVPGSDVT ILNVLMNPTR
351 TGLILTLQEM GADIEVINPR LAGGEDVADL RVRSSTLKGV TVPEDRAPSM
401 IDEYPILAVA AAFAEGATVM NGLEELRVKE SDRLSAVANG LKLNGVDCDE
451 GETSLVVRGR PDGKGLGNAS GAAVATHLDH RIAMSFLVMG LVSENPVTVD
501 DATMIATSFP EFMDLMAGLG AKIELSDTKA A
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C. Chloroplast Transit Peptide (CTP2)

The target for glyphosate in plants, the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, is located in the chloroplast. Many chloroplast-localized proteins, including EPSPS, are expressed from nuclear genes as precursors and are targeted to the chloroplast by a chloroplast transit peptide (CTP) that is removed during the import process. It has been demonstrated *in vivo* (Timko et al., 1988) and *in vitro* (Della-Cioppa et al., 1986 and 1987) that non-chloroplast proteins may be targeted to the chloroplast by use of protein hybrids containing a CTP and that a CTP amino acid sequence is sufficient to target a protein to the chloroplast. To achieve chloroplast localization of the CP4 EPSPS protein, which, as a bacterial protein, contains no CTP, the *ctp* coding sequence from the *Arabidopsis thaliana epsps* coding region (Klee et al., 1987) was joined to the *cp4 epsps* coding sequence. To accomplish this, the *Arabidopsis ctp* DNA sequence was modified by site-directed mutagenesis to place a *Sph*I restriction site at the CTP processing site. This change replaced the Glu-Lys at this location with Cys-Met. The DNA sequence of this CTP peptide is designated as *ctp2*. The CTP2::CP4 EPSPS hybrid protein was demonstrated to allow import into chloroplasts isolated from *Lactuca sativa*, using methods described previously (Della-Cioppa et al., 1986 and 1987). Other Roundup Ready crops that have been previously reviewed and granted nonregulated

status by the USDA, including cotton (95-045-01p), corn (96-317-01p) and sugar beet event 77 (98-173-01p), contain this same *ctp2::cp4 epsps* DNA construct.

D. Regulatory Sequences

P-FMV promoter: The initiation of transcription of the *ctp2::cp4 epsps* coding region is controlled by the 35S gene promoter. This promoter, from a modified figwort mosaic virus (FMV), is constitutively active in plants (Sheperd et al., 1987; Richins et al., 1987; Gowda et al., 1989; Sanger et al., 1990). It is contained on a 0.672 kb DNA fragment located 5' to the *ctp2::cp4 epsps* coding region (see Table IV-1 and Figure IV-1). This same promoter was used to control transcription of the same coding region in other Roundup Ready crops that have been previously reviewed and granted nonregulated status by the USDA, including cotton (95-023-01p), sugar beet event 77 (98-173-01p) and canola (98-216-01p).

E9 3' polyadenylation sites: Polyadenylation sites direct mRNA processing and multiple adenylate addition. The P-FMV::*ctp2::cp4 espsp*::E9 3' gene cassette contains a 0.63 kb DNA fragment from the 3' end of the *Pisum sativum* (pea) *rbc*S E9 gene to provide these polyadenylation sites (Coruzzi et al., 1984; Morelli et al., 1985); also see Table IV-1 and Figure IV-1. This same DNA region was used as the polyadenylation signal for the same coding region in other Roundup Ready crops that have been previously reviewed and granted nonregulated status by the USDA, including cotton (95-023-01p), sugar beet event 77 (98-173-01p) and canola (98-216-01p).

E. T-DNA Border Sequences

Plasmid vector PV-BVGT08 contains DNA sequences that are necessary for the transfer of T-DNA into the plant cell. These are termed the Right Border (RB) and Left Border (LB) regions, and each region contains a 21-25 bp sequence that defines the extent of DNA that can be transferred into the plant genome. The RB region is a 365 bp nucleotide sequence that was originally isolated from *A. tumefaciens* plasmid pTiT37 (Depicker et al., 1982). It corresponds to nucleotides 1676-2042 in the GenBank sequence for the *A. tumefaciens* strain C58 Ti plasmid sequence (GenBank accession AE007928). In PV-BVGT08 it is located 5' to the P-FMV::*ctp2::cp4 epsps*::E9 3' gene cassette (nucleotide positions 7601-7965 on the PV-BVGT08 map), that is, nearest to the P-FMV promoter (see Figure IV-1). The LB region is a 459 bp nucleotide sequence that was originally isolated from *A. tumefaciens* plasmid pTI15955, a derivative of plasmid pTiA6 (Barker et al., 1983). It corresponds to nucleotides 193986-194140 and 1-305 in the GenBank sequence for the *A. tumefaciens* strain A6 Ti plasmid sequence (GenBank accession AR242881). In PV-BVGT08 it is located 3' to the P-FMV::*ctp2::cp4 epsps*::E9 3' gene cassette (nucleotide positions 2403-2862 on the PV-BVGT08 map), that is, nearest to the E9 3' polyadenylation signal.

F. Genetic Elements Outside the T-DNA Border Sequences

The elements described below are present on plasmid vector PV-BVGT08 but are outside the border sequences of the T-DNA. Hence, they were not expected to be transferred into the sugar beet genome, as confirmed by data presented in Section V.A.2.

ori-V: A 0.393 kb DNA fragment, originally isolated from plasmid RK2, containing a vegetative origin of DNA replication that allows maintenance of the plasmid in *Agrobacterium*.

ori-322: A 0.629 kb DNA fragment containing an additional origin of DNA replication that allows maintenance of the plasmid in other bacteria, such as *E. coli*.

rop: A 0.191 kb DNA fragment, originally isolated from plasmid pBR322, which represses the formation of RNA primer, and is critical to providing maintenance and copy number control of plasmids in bacterial hosts, such as *E. coli*.

aad: A 0.789 kb DNA fragment from the bacterial transposon Tn7 containing the gene that encodes for the streptomycin adenyltransferase enzyme and allows selection of bacteria on culture media containing streptomycin or spectinomycin.

V. GENETIC ANALYSIS AND MOLECULAR CHARACTERIZATION

A. Molecular Characterization of Event H7-1

Molecular analysis was performed to characterize the DNA inserted in event H7-1. Genomic DNA was analyzed using Southern blot analysis (Southern, 1975) to determine the insert number (number of integration sites within the sugar beet genome); the copy number (number of DNA segments used for transformation integrated within one insertion site); the integrity of the inserted promoter, coding region, and polyadenylation sequence; and the presence or absence of the plasmid backbone sequence. Polymerase chain reaction (PCR) (Saiki, 1990) was performed to verify the sequences at the 5' and 3' ends of the insert.

These analyses support the following conclusions: (1) the genome of event H7-1 contains a single DNA insertion comprised of a single copy of the *cp4 epsps* gene expression cassette used for transformation; (2) the *cp4 epsps* gene expression cassette within the single insert is intact; (3) transcription of the *cp4 epsps* gene expression cassette contains the 35S gene promoter from a modified figwort mosaic virus genome (P-FMV), which directs the transcription of the coding sequence for a chloroplast transit peptide from *Arabidopsis thaliana* (*ctp2*), which allows post-translational transport into the chloroplast and is fused to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) coding sequence from *Agrobacterium* sp. strain CP4 (*cp4 epsps*), and DNA containing polyadenylation sequences from the 3' nontranslated region of the *Pisum sativum* (pea) *rbc*S E9 gene (E9 3'); and (4) the genome of event H7-1 does not contain any detectable plasmid backbone DNA.

A.1. Materials and methods

A.1.a. Test substance. The test substance for molecular characterization was event H7-1. DNA was isolated from cells of the original transformant, identified as line 6401VH, and from three additional progenies identified as lines 64801H, 74922H and 83002S, respectively (see Table III-1).

A.1.b. Control substances. The primary control substance was the conventional sugar beet line 3S0057, the line that was used for the transformation experiments. Control line 3S0057 was used as the nontransgenic control in Figures V-2, V-3, V-4, V-5 and V-7. Additional control substances were the sugar beet genotypes 5R7150, 8K1180 and 6S0085. These additional control substances were used as controls in Figure V-11. These lines are common, nontransgenic lines used for conventional sugar beet breeding.

A.1.c. Reference substances. The reference substance was the plasmid used for the transformation, PV-BVGT08. Plasmid DNA and DNA from the control sugar beet line were mixed together, digested with restriction enzymes and separated by electrophoresis on agarose gels in parallel to the test substance. The plasmid served as a size marker for the expected fragment and as a positive hybridization control. The plasmid DNA was

mixed with the genomic plant DNA at a concentration representing less than one copy of the element being analyzed to demonstrate the sensitivity of the Southern method (~10 μg genomic DNA and ~28 pg PV-BVGT08 DNA). For size estimations, the molecular size marker RAOUL (ONCOR/Appligene, catalog #160673) was used as a reference substance.

A.1.d. DNA isolation.
Method I. Plant tissue (1-3 g fresh weight) was ground frozen in liquid nitrogen to a fine powder using a mortar and pestle. The powder was transferred to a 50 ml Oakridge tube, and 7.5 ml of preheated (60°C) CTAB buffer (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA, pH 8.0, 1.4 M NaCl and 0.2% mercaptoethanol) were added. The samples were incubated at 65°C for approximately 30 minutes with intermittent mixing. An equal volume (8 ml) of a mixture of room temperature (RT) chloroform:isoamyl alcohol (24:1) was added to the samples. The suspension was mixed by inversion, and the two phases were separated by centrifugation (10 minutes, 9000 rpm). The aqueous phase was transferred to a new 50 ml Oakridge tube, followed by precipitation of the DNA by addition of 5 ml isopropanol. The DNA was pelleted by centrifugation (two minutes, 9000 rpm) and the supernatant was removed. The precipitated DNA was incubated with a wash solution of 76% ethanol and 10 mM ammonium acetate for about 20 minutes. After centrifugation and decanting of the supernatant, the DNA was vacuum dried and redissolved in TE buffer, pH 8.0, at 4°C overnight.

Method II. As an alternative method, DNA was isolated using the DNeasy Plant Maxi Kit from Qiagen (catalog #68163). DNA isolation was carried out according to the manufacturer's manual.

A.1.e. DNA quantitation and restriction enzyme digestion. DNA quantification was performed using a LKB Biochrom UV/visible spectrophotometer or, alternatively, after agarose gel electrophoresis by scanning the DNA with the RFLPscan program (MWG-Biotech). As a calibration standard, the High DNA Mass Ladder from Gibco/Life Technologies (catalog # 10496-016) was used. Approximately 10 μg of genomic DNA from the test and the control lines was used for the restriction enzyme digests. Overnight digests were performed according to the manufacturer's manual in a total volume of 250 μl using 100 units of the restriction enzyme. Restriction enzymes were purchased from Boehringer Mannheim, Stratagene or New England Biolabs.

A.1.f. DNA probe preparation. PV-BVGT08 DNA was isolated from *E. coli* cultures. Probe templates homologous to the *cp4 epsps* coding region, the FMV promoter (P-FMV), the E9 3' polyadenylation signal, the P-FMV::*ctp2*::*cp4 epsps*::E9 3' gene cassette (Figure V-1), and the backbone regions were prepared by digests with the corresponding restriction enzymes, followed by separation on agarose gel or by polymerase chain reaction (PCR). The products were purified using the Geneclean II Kit of BIO 101 (La Jolla, CA). Labeling of the probes (25 pg) with ^{32}P-dCTP or ^{32}P-dATP was achieved by making use of the Megaprime DNA labeling system of Amersham-Pharmacia Biotech Europe.

A.1.g. Southern blot analyses. The samples of DNA treated with restriction enzymes were separated by agarose gel electrophoresis for ~15 hours at ~35 volts. After photographing the gel, the DNA was depurinated by soaking the gel for 15 minutes in a 0.25 M HCl solution, denatured by incubating the gel for 30 minutes in a denaturing solution of 0.5 M NaOH and 1.5 M NaCl with constant gentle agitation and, finally, neutralized by soaking for two hours in several volumes of a solution of 2 M NaCl and 1 M Tris-HCl, pH 5.5. The DNAs from the agarose gels were transferred to Hybond-N nylon membranes (Amersham Pharmacia Biotech Europe) using a PosiBlot Pressure Blotter from Stratagene according to the manufacturer's directions. After soaking the filter for 15 minutes in 2X SSPE (SSPE is 3.6 M NaCl, 20 mM EDTA, 0.2 M NaH_2PO_4/Na_2HPO_4, pH 7.4), the DNA was fixed to the membrane by illumination with UV-light (Transilluminator Pharmacia) for one minute and by baking for one hour at 80°C in a vacuum oven. The blots were prehybridized for four hours in an aqueous solution of 50% formamide, 5X SSC, 0.1% laurylsarcosine, 0.02% SDS and 2% blocking reagent (Boehringer Mannheim, catalog # 1096176). Hybridization with the radiolabeled probe was done in fresh prehybridization solution for 16 to 18 hours at 42 °C. After hybridization, membranes were washed for five minutes in 2X SSC at 42°C, for 20 minutes in 2X SSC, 1% SDS at 65°C , and for two 15-minute periods in 0.2X SSC, 0.1% SDS at 68°C. Autoradiographic images of the blot were obtained by exposing the blots using Kodak Biomax MS film in conjunction with Kodak Biomax MS intensifying screens.

A.2. Results and discussion

Figure V-1 shows a diagram of the DNA insert in event H7-1, along with the probes and relevant restriction endonuclease sites, which is provided as an aid in interpreting the results presented in the following sections.

A.2.a. Insert number. The number of integration sites of event H7-1 DNA into the sugar beet genome was evaluated. In order to determine the insert number, the genomic DNA was digested with the restriction enzymes *Hind*III, *Xba*I and *Bam*HI. As a negative control, DNA from the control plant representing the same genetic background was digested with *Bam*HI. As a positive control, transformation vector DNA (PV-BVGT08) was used.

*Xba*I and *Bam*HI cleave only once in PV-BVGT08 and do not cleave inside the labeled *cp4 epsps* probe used (Figure IV-1 and Figure V-1). *Hind*III cleaves three times in PV-BVGT08, but all three sites are located outside of the probe and on the same side, 5' relative to the probe. Thus, each enzyme should release a single DNA fragment that hybridizes to the *cp4 epsps* probe and contains a part of the inserted DNA and adjacent plant genomic DNA. The number of fragments detected indicates the number of inserts present in event H7-1.

The results are shown in Figure V-2. After digestion with the enzymes *Hind*III, *Xba*I or *Bam*HI, only a single hybridization fragment was detected. The fragments of 5.2 kb from the *Hind*III digest (lane 4), 4 kb from the *Xba*I digest (lane 5) and approximately 11 kb from the *Bam*HI digest (lane 7) showed that event H7-1 represents a single integration event (Figure V-1). The hybridization of the plasmid PV-BVGT08 with the *cp4 epsps* probe resulted in a 8.6 kb signal (Figure V-2, lane 1), as expected. A second smaller, very faint band is likely due to the incomplete digestion of PV-BVGT08. This assessment is based on the knowledge that plasmids may appear in different forms (isoforms), e.g., circular, linear or multimeric as concatamers. Incomplete digestion of a highly concentrated sample of plasmid DNA applied to an agarose gel can result in one

Figure V-1. Diagram of DNA insert in event H7-1

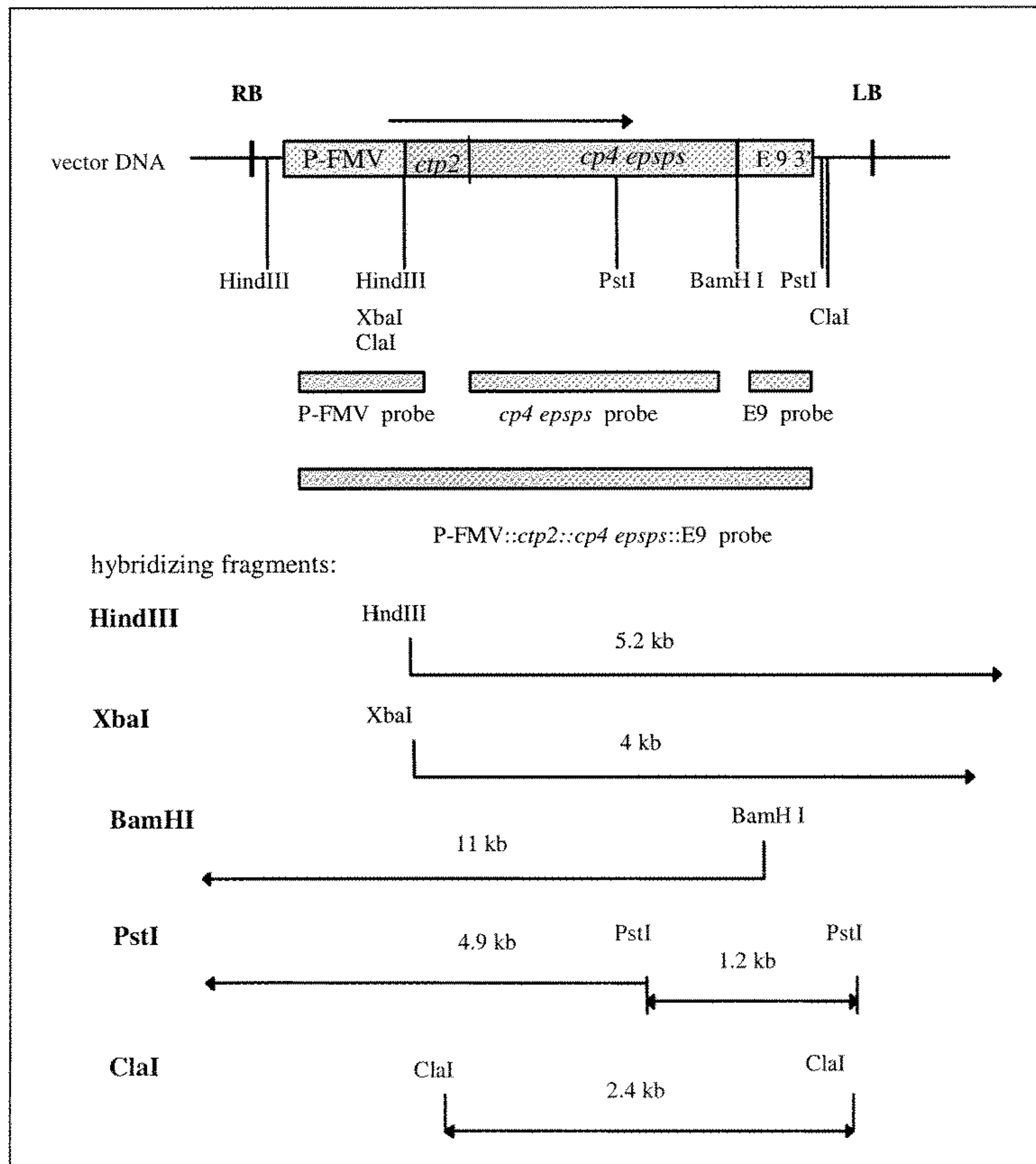
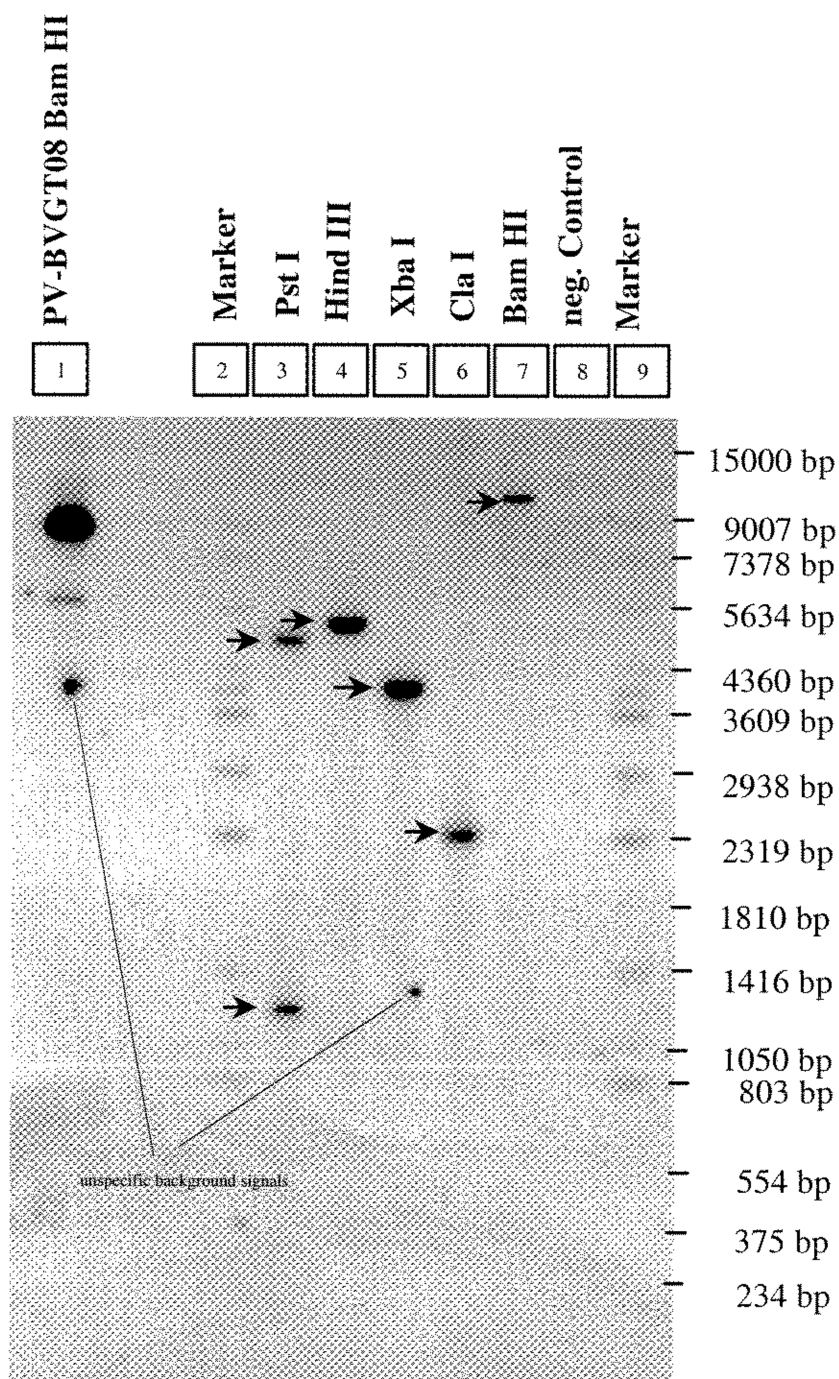


Figure V-2. Southern blot analysis of event H7-1: Insert and copy number analysis



Legend: 10 µg of event H7-1 genomic DNA were digested with *Pst*I, *Hind*III, *Xba*I, ClaI and *Bam*HI (lanes 3 to 7). Control sugar beet genomic DNA as a negative control was digested with *Bam*HI (lane 8). Plasmid PV-BVGT08 as a positive control was digested by *Bam*HI (lane 1). Lanes 2 and 9 contain size makers. The blot was probed with a ^{32}P-labeled *cp4 epsps* coding region fragment. The probe is an internal sequence of the *cp4 epsps* coding region covering basepairs 447 to 1555.

or more bands observed. This is concluded to be the case in Figure V-2. The strong signal in Lane 1 represents the linearized PV-BVGT08 plasmid, whereas the faint band is one of the incompletely digested isoforms.

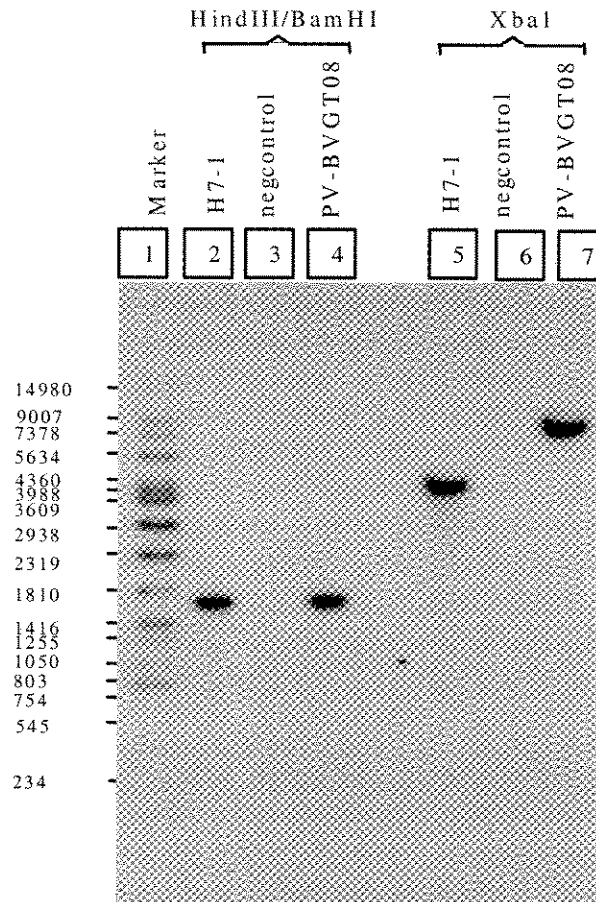
A.2.b. Copy number. In theory, one integration site could contain more than one copy of the inserted DNA. However, this is unlikely based on the sizes of the fragments obtained in the restriction digests described above. If more than one copy of the inserted DNA were present in event H7-1, additional fragments would likely be detected. This was confirmed by a digest with restriction enzyme *Pst*I. *Pst*I cleaves twice in the T-DNA, between the Left Border and Right Border sequences (Figure V-1). One of the restriction sites is within the *cp4 epsps* coding region, so after digestion with *Pst*I, one would expect to detect two hybridizing fragments with the *cp4 epsps* probe. One of the expected fragments should correspond to an internal fragment of about 1.2 kb, and the second fragment should contain a region of fusion with the sugar beet genome. Again, if there were more than one copy, additional fragments should be detected. The result of this digestion with *Pst*I showed that *Pst*I cuts the DNA as expected. The internal fragment of 1.2 kb and only one additional fragment of about 4.9 kb were detected (Figure V-2, lane 3), confirming that there is only one copy of the inserted DNA in event H7-1.

As an additional internal control, the DNA from event H7-1 was cleaved with *Cla*I (see Figure V-1). *Cla*I cleaves twice within the inserted P-FMV*::ctp2::cp4 epsps::*E9 3' gene cassette (Figure V-2, lane 6). As predicted, only a single fragment of 2.4 kb hybridizes with the *cp4 epsps* probe. This result also demonstrates the intactness of the integrated DNA fragment.

A.2.c. Integrity of inserted P-FMV*::ctp2::cp4 epsps::*E9 3' gene cassette. The integrity of the *cp4 epsps* gene cassette, with respect to the individual elements (P-FMV promoter, *cp4 epsps* coding region, and E9 3' nontranslated region), was assessed by digestion with the enzymes *Hind*III for P-FMV, *Hind*III plus *Bam*HI for *cp4 epsps*, and *Eco*RI plus *Pst*I for the E9 3' nontranslated region. Additional experiments were performed with *Sac*I plus *Xho*I for the P-FMV*::ctp2::cp4 epsps* region and for the E9 3' region. Plasmid DNA mixed with control sugar beet DNA and control sugar beet DNA alone were digested with the same enzymes, as positive and negative controls, respectively. These enzymes cleave within the intended DNA insert, between the Left and Right T-DNA Borders (see the plasmid map in Figure IV-1). If the respective elements are intact, the size of the hybridized fragments should be identical in event H7-1 DNA and PV-BVGT08 DNA.

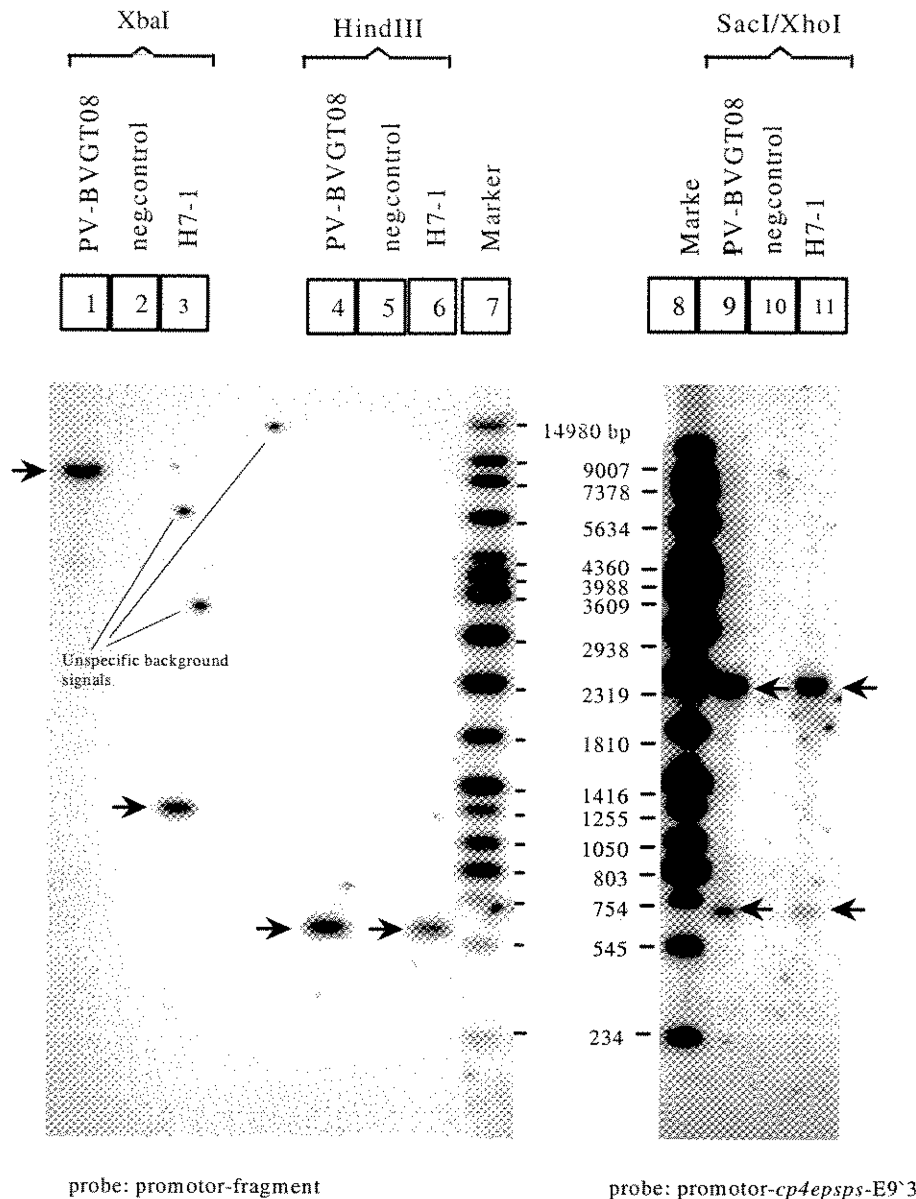
As additional evidence of intactness, event H7-1 and plasmid DNAs were digested with *Xba*I. *Xba*I cleaves once within the plasmid vector, between the promoter and the *ctp2::cp4 epsps* coding region (Figure V-1). Therefore, one would expect with PV-BVGT08 DNA a 8.6 kb band, corresponding to the size of the complete plasmid, and with event H7-1 DNA an insert-genomic DNA junction fragment which differs in size compared to the PV-BVGT08 DNA band. The results of these analyses are shown in Figures V-3, V-4 and V-5.

Figure V-3. Southern blot analysis of event H7-1: *ctp2::cp4 epsps* coding region intactness



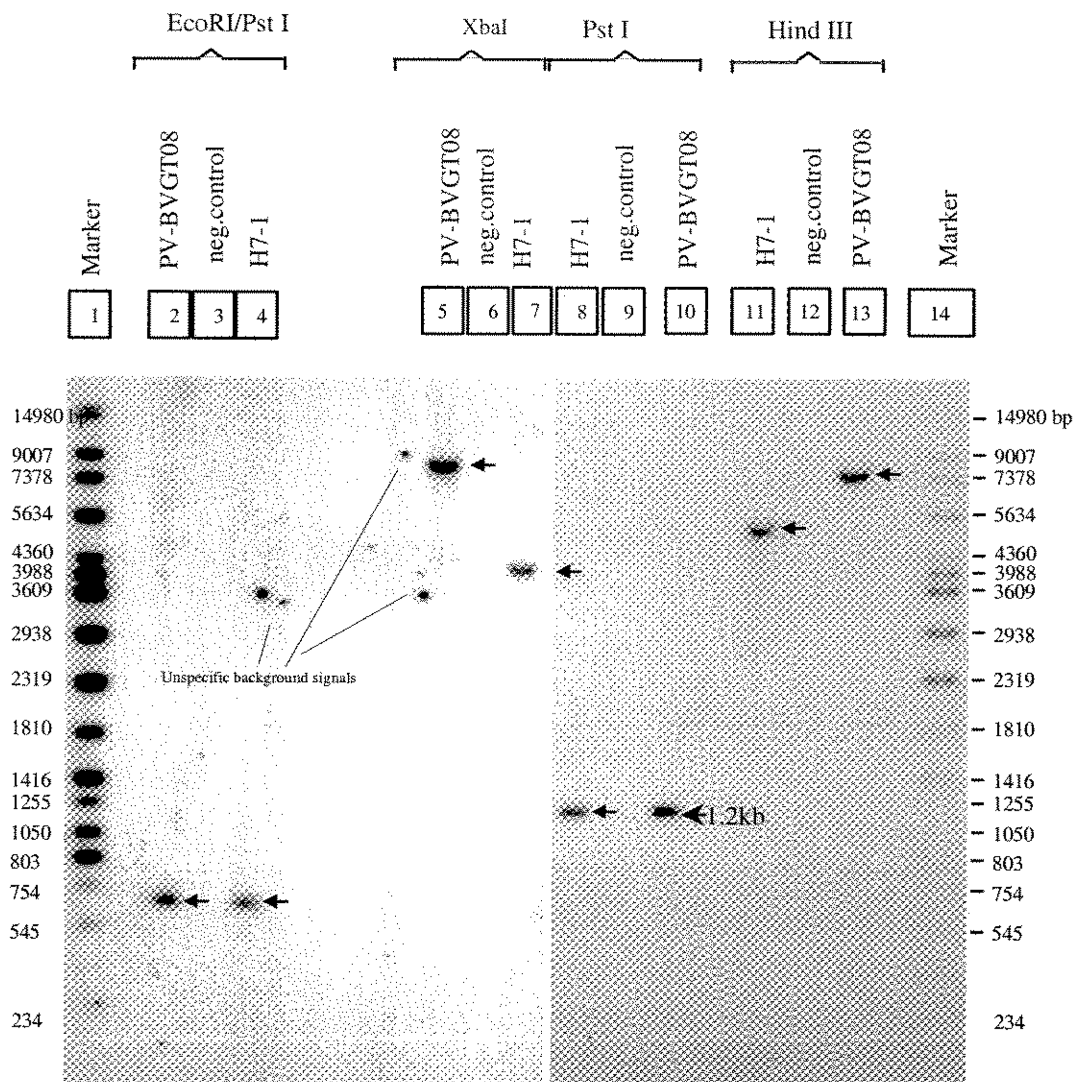
Legend: 10 μg of event H7-1 genomic DNA, control DNA or control DNA mixed with PV-BVGT08 were digested with *Xba*I (lanes 5, 6 and 7) or *Hind*III plus *Bam*HI (lanes 2, 3 and 4). The blot was probed with a ^{32}P-labeled *cp4 epsps* (PCR) fragment. The probe represents the sequence of PV-BVGT08 from bp 447 to 1555.

Figure V-4. Southern blot analysis of event H7-1: Promoter region intactness



Legend: 10 μg of event H7-1 genomic DNA, control DNA or control DNA mixed with PV-BVGT08 were digested with *Hind*III (lanes 4, 5 and 6), *Xba*I (lanes 1, 2 and 3), and *Sac*I plus *Xho*I (lanes 9, 10 and 11). The blot was probed with a ^{32}P-labeled promoter fragment (from *Hind*III digestion, equal to the PV-BVGT08 sequence from bp 7972 to 8583) or with the complete P-FMV::*ctp2*::*cp4 epsps*::E9-3' cassette (from *Pme*I plus *Xho*I digestion, equal to the PV-BVGT08 sequence from bp 7935 to 2389).

Figure V-5. Southern blot analysis of event H7-1: Polyadenylation signal intactness



Legend: 10 μg of event H7-1 genomic DNA, control DNA, or control DNA mixed with PV-BVGT08 were digested with *Eco*RI plus *Pst*I (lanes 2, 3 and 4), or *Xba*I (lanes 5, 6 and 7), *Hind*III (lanes 11, 12 and 13), or *Pst*I alone (lanes 8, 9 and 10). The blot was probed with a ^{32}P-labeled E9 3' polyadenylation fragment from *Bam*HI plus *Xho*I digestion, equal to the PV-BVGT08 sequence from bp 1702 to 2389.

In Figure V-3, the enzymatic digestions with *Hind*III and *Bam*HI released the *ctp2::cp4 epsps* coding region and the blot was probed with a *cp4 epsps* fragment generated by PCR. The negative control (lane 3) did not show any hybridization bands. Genomic DNA from event H7-1 (lane 2) and plasmid PV-BVGT08 mixed with control sugar beet DNA (lane 4) both produced an approximately 1.7 kb band, which is the expected size. The digest with *Xba*I resulted in the expected 8.6 kb band of the linearized PV-BVGT08 (lane 7). For event H7-1 the digest resulted in an insert-genomic DNA junction fragment of approximately 4.0 kb (see also Figure V-1 and Figure V-3, lane 5). Again, the negative control did not produce a signal (Figure V-3, lane 6).

In Figure V-4, the enzymatic digestion with *Hind*III released the P-FMV promoter, which was probed with a promoter fragment generated by PCR. The negative control (lane 5) did not show a hybridization signal. Genomic DNA from event H7-1 (lane 6) and plasmid PV-BVGT08 spiked into control sugar beet DNA (lane 4) both produced a hybridizing fragment of approximately 0.6 kb in size. These fragments correspond to the expected size of the P-FMV promoter fragment.

The digest with *Xba*I resulted in the predicted 8.6 kb band from the linearized plasmid (lane 1) and an insert-genome DNA junction fragment of approximately 1.3 kb from event H7-1 (lane 3). The presence of the 1.3 kb fragment is also additional evidence that event H7-1 contains only a single copy of the P-FMV*::ctp2::cp4 epsps::*E9 3' gene expression cassette. Again, the negative control (lane 2) did not produce a signal.

The digest with *Sac*I and *Xho*I released two fragments, one of which contained the P-FMV promoter together with the *cp4 epsps* coding region, and another which contained the E9 3' polyadenylation signal. Hybridization with the complete P-FMV*::ctp2::cp4 epsps::*E9 3' polyadenylation signal cassette probe (from a *Pme*I plus *Xho*I digestion) produced the predicted 2.3 kb P-FMV::*cp4 epsps* hybridization band and the 0.7 kb E9 3' polyadenylation signal band from the plasmid DNA spiked into control sugar beet DNA (lane 9) and event H7-1 genomic DNA (lane 11).

In Figure V-5, the enzymatic digestions with *Pst*I and *Eco*RI released a fragment that hybridized with the E9 3' polyadenylation signal probe. The negative control (lane 3) did not show hybridization bands. Plasmid DNA spiked into control sugar beet DNA (lane 2) and the genomic DNA from event H7-1 (lane 4) each produced a hybridization band of approximately 0.6 kb, which corresponds to the predicted size for the E9 3' fragment.

The enzymatic digestion with *Xba*I resulted in an expected 8.6 kb hybridization band from the linearized plasmid (lane 5) and an approximately 4.0 kb insert-genomic DNA junction fragment from event H7-1 (lane 7), confirming the presence of a single copy of the insert.

The digest with *Pst*I released a fragment that contained the E9 3' polyadenylation signal plus a portion of the 3' end of the *cp4 epsps* coding region and was probed with the same

E9 3' polyadenylation signal. The resulting 1.2 kb fragment was detected, as predicted, in both the event H7-1 genomic DNA (lane 8) and the plasmid DNA (lane 10).

Digestion with *Hind*III resulted in a 8.0 kb band from the linearized plasmid minus the promoter sequences (lane 13) and a 5.2 kb insert-genomic DNA junction fragment from event H7-1 genomic DNA (lane 11). The single 5.2 kb fragment from the *Hind*III digest and the single 4.0 kb fragment from the *Xba*I digest also substantiate that event H7-1 contains only one copy of inserted DNA, as the negative controls did not produce a signal (lane 12).

A.2.d. Analysis for genetic elements from outside the T-DNA border sequences. Genetic elements from outside of the border sequences of the T-DNA on plasmid PV-BVGT08 (see Table IV-1 and Figure IV-1) were not expected to be transferred into the sugar beet genome. The following experiments were performed to confirm that these elements (*ori-V, ori-322* and *aad)* were not inserted into the genome of event H7-1.

To determine the absence of backbone DNA in event H7-1, genomic DNA from event H7-1, control sugar beet and genomic DNA from event H7-1 spiked with plasmid DNA were digested with the restriction enzyme *Xba*I, then probed with three overlapping PCR-generated probes that encompass the entire backbone sequence. A fourth probe was also employed, which consisted of the entire backbone in one fragment (Figure V-6).

The probes correspond to the following backbone sequences:

- Probe 1: bp 2730-5370
- Probe 2: bp 5278-6419
- Probe 3: bp 6302-7851
- Probe 4: bp 2730-7851

Figure V-6. Diagram of backbone probes

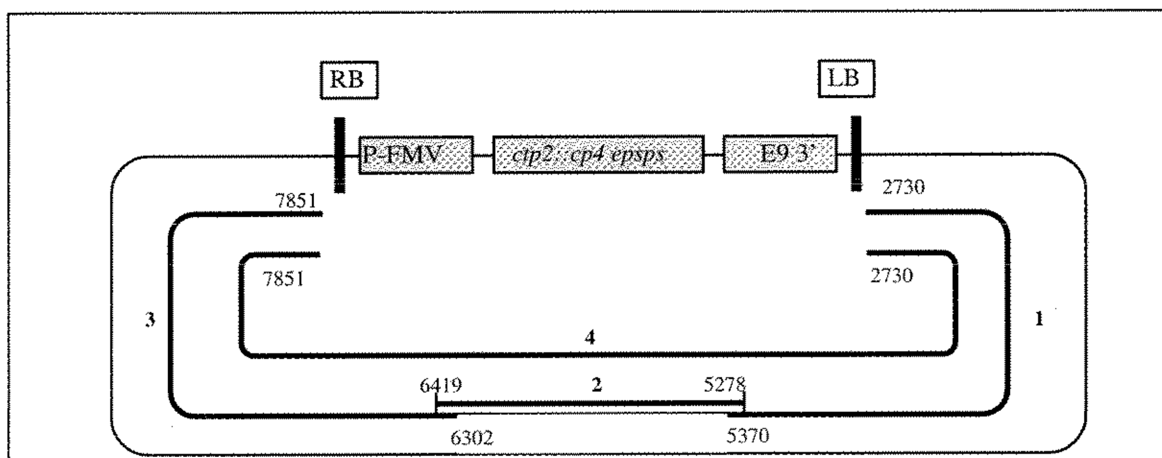


Figure V-7 shows the result of the Southern blot analyses employing the four probes to determine absence of backbone. In lanes 6, 10, 14 and 18, digestion of event H7-1 genomic DNA, probed with the overlapping backbone probes, did not show hybridization bands. Only the positive controls, lanes 4, 8, 12, 16 and 20, with event H7-1 genomic DNA spiked with PV-BVGT08 DNA showed bands of 8.6 kb, as expected. These bands represent the linearized PV-BVGT08 DNA.

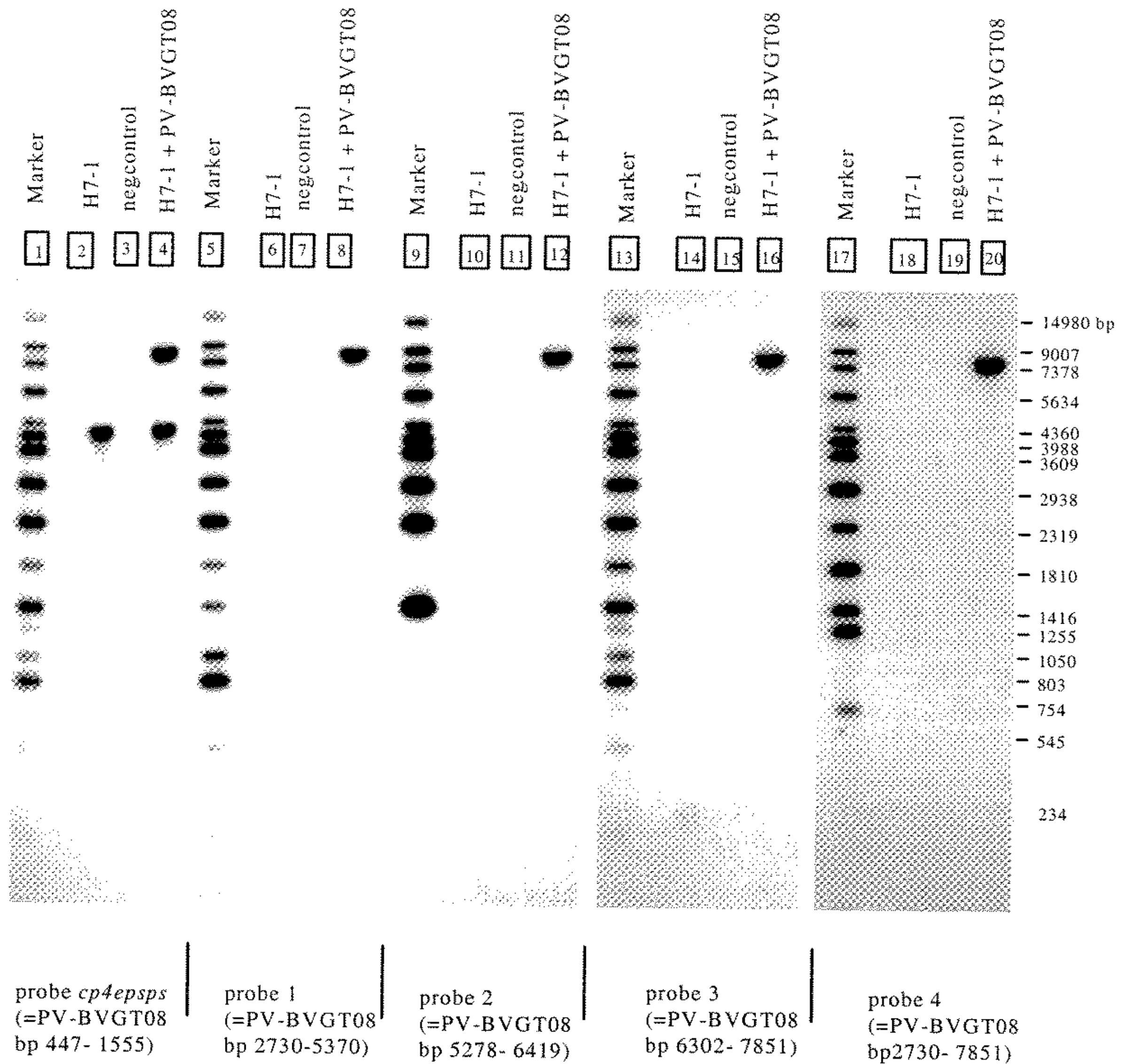
As a DNA concentration control, event H7-1 genomic DNA (lane 2) and event H7-1 genomic DNA spiked with PV-BVGT08 DNA (Figure V-7, lane 4), both digested with *Xba*I, were hybridized with a probe that contained the *cp4 epsps* coding region. The 4.0 kb band in lane 2 represents a DNA fragment that contains the promoter fused to a portion of sugar beet genomic DNA. The two bands of lane 4 represent this same 4.0 kb fragment plus the 8.6 kb linearized PV-BVGT08 plasmid DNA. Both bands have the same intensity. This is a clear indication that the concentration of the added PV-BVGT08 DNA is comparable to the concentration of the *cp4 epsps* element in the event H7-1 DNA. The concentration of plasmid DNA used was equivalent to 0.5 copy. If there were significant backbone sequences integrated in the event H7-1 genome, clear signals should be detectable.

A.2.e. Identification of 5' and 3' genomic flanking sequences. *Agrobacterium*-mediated transformation normally leads to the integration of all sequences between the Left and Right Borders into the plant genome. The ends of the integrated plasmid DNA should be within or near the *A. tumefaciens* Right Border and the *A. tumefaciens* Left Border sequences. Therefore, an inverse-PCR technique was used to identify those regions. The cloned PCR products were sequenced and the sequence data compared to plasmid PV-BVGT08 sequence.

Figure V-8 shows the alignment of the sequence from the cloned inverse PCR fragment, obtained with primers aligned with the Left Border region, compared to the plasmid sequence. The comparison of these sequences demonstrated that the homology stopped exactly within the Left Border sequence with 21 nucleotides of the Left Border inserted.

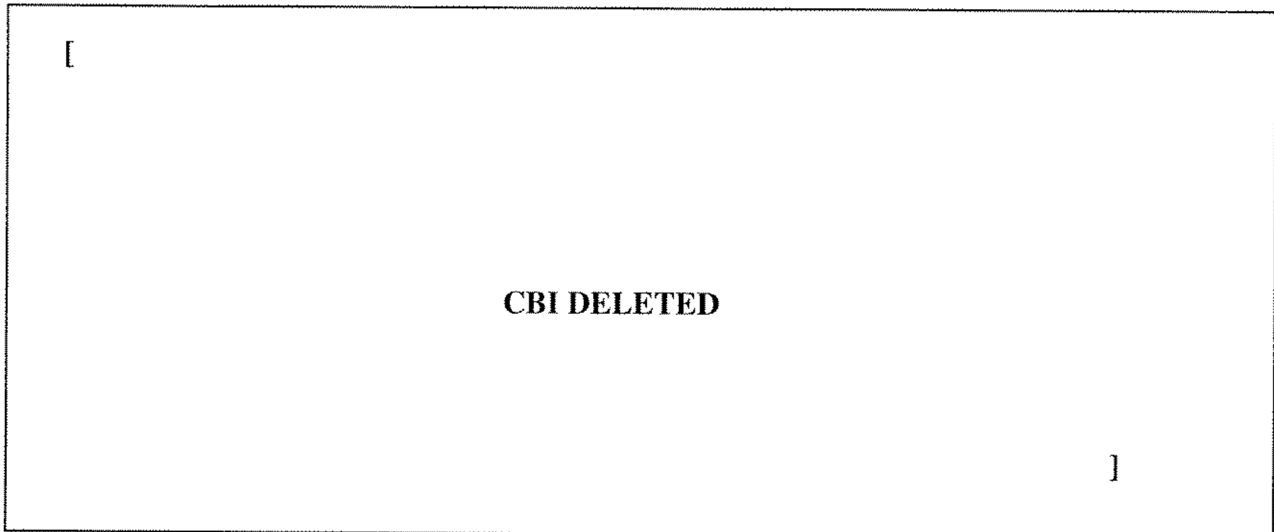
Figure V-9 shows the alignment of the sequence from the cloned inverse-PCR fragment, obtained with primers aligned with the Right Border region, compared to the plasmid sequence. The comparison of these sequences showed that the homology was interrupted 18 nucleotides in front of border sequence, such that no Right Border sequences are contained in event H7-1.

Figure V-7. Southern blot analysis of event H7-1: Backbone analysis



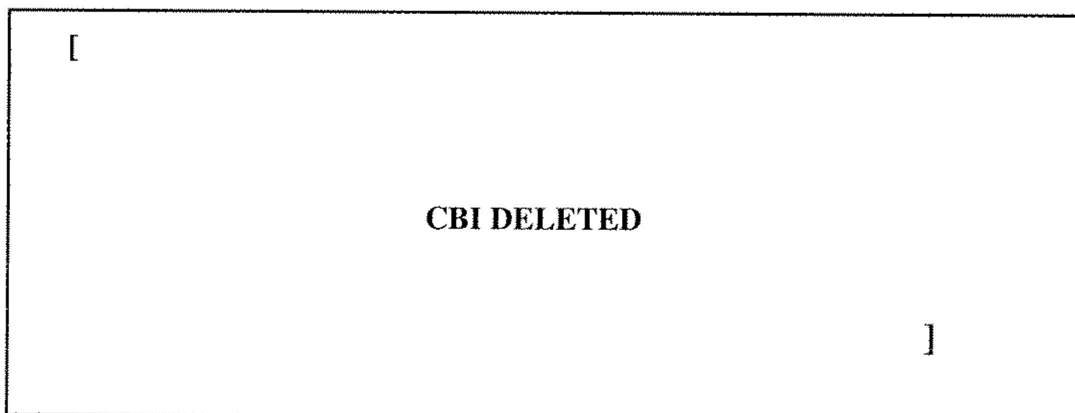
Legend: 10 μg of event H7-1 genomic DNA, control DNA, and event H7-1 genomic DNA mixed with PV-BVGT08 were digested with *Xba*I. The blots were probed with ^{32}P-labeled probes encompassing the entire backbone of PV-BVGT08 (probes 1-4). One blot was probed with a labeled *cp4 epsps* coding region fragment.

Figure V-8. Comparison between the PCR fragments and the PV-BVGT08 sequences on the Left Border region



Legend: In the lower sequence from PV-BVGT08, the Left Border (LB) sequence is underlined and bold. In the upper sequence from event H7-1, the plant genome sequence is underlined and bold. The arrow indicates the insert-plant DNA fusion point in event H7-1.

Figure V-9. Comparison between the PCR fragments and the PV-BVGT08 sequences on the Right Border region



Legend: In the lower sequence from PV-BVGT08, the Right Border (RB) sequence is underlined and bold. In the upper sequence from event H7-1, the genomic plant DNA is underlined and bold. The arrow indicates the insert-plant DNA fusion point in event H7-1.

A.3. Conclusions

Detailed molecular characterization studies have shown that a single T-DNA insert is detected in the genome of event H7-1. This insert contains one copy of the P-FMV*::ctp2::cp4 epsps::*E9 3' gene expression cassette and contains no detectable backbone sequence from the plasmid used for the transformation. In particular, the origins of replications and the *aad* gene are not present in event H7-1.

The junction of the integrated DNA, relative to the Right Border of the T-DNA, is at base pair 7939 of plasmid PV-BVGT08 (Figures V-9 and V-10); thus, no Right Border sequences are contained with the integrated DNA. The junction of the integrated DNA, relative to the Left Border of the T-DNA, is at base pair 2703 (Figures V-8 and V-10), and the integrated DNA contains a small amount (21 nucleotides) of Left Border sequences. Therefore, the molecular size of the T-DNA inserted into the genome of event H7-1 is approximately 3.4 kb.

The results of the molecular characterization studies are summarized in Table V-1.

Table V-1. Summary of event H7-1 insert analysis

Genetic Element	Copy Number
P-FMV*::ctp2::cp4 epsps::*E9 3' cassette	one copy
ori-V	not present
ori-322	not present
aad	not present
other backbone sequences outside the T-DNA	not present

The DNA insert in the genome of Roundup Ready sugar beet event H7-1, compared to plasmid PV-BVGT08, is shown schematically in Figure V-10. The molecular data (Figures V-2, V-3, V-4 and V-5), as well as the Mendelian inheritance data (Table V-2), also support the conclusion that a single stable insertion is present in event H7-1 and that this insertion contains one complete copy of the *cp4 epsps* gene cassette inserted into the genomic DNA of sugar beet.

Figure V-10. Diagram of T-DNA in plasmid PV-BVGT08 and in the genome of event H7-1

B. Segregation Data and Stability of Gene Transfer of Event H7-1

B.1. Segregation data

In other glyphosate-tolerant commercial crops (e.g., Roundup Ready soybean, Roundup Ready canola and Roundup Ready cotton), glyphosate tolerance is inherited as a dominant trait in a Mendelian manner. For sugar beet, numerous hybrid crosses and multiplications (using conventional breeding techniques) with event H7-1 were conducted (Figure III-2). The inheritance of the introduced DNA in the progenies from these crosses or multiplications was monitored phenotypically at the whole plant level by application of a Roundup agricultural herbicide at the two-leaf stage in greenhouse experiments. Data from these analyses provide further evidence of the number of loci, as well as the stability of the introduced DNA. The results from the analysis of the segregation patterns of event H7-1 are presented in Table V-2. The segregation patterns observed for event H7-1 across multiple breeding experiments, representing four generations, indicate no statistical significance by Chi-square analysis in all but three instances. In a first generational breeding experiment (64038G), the segregation pattern was not 1:1 as expected, which was attributed to the small population of plants tested.

Table V-2. Segregation pattern with event H7-1

Generation	Identifier	Pedigree[1]				No. plants	% Tolerant plants[3]		Chi-square test		Comment
		Female[2]		Male			Observed	Expected	Chi test value	Sig.[4]	
1	64037G	3A0047	MO	6401VH	H7-1	62	51.6	50	0.10	NS	
1	64038G	3A0064	MO	6401VH	H7-1	44	63.6	50	7.40	*	Few tested plants [6]
1	64801H	6401VH	H7-1	6401VH	H7-1	77	67.5	75	3.00	NS	
2	74022G	4E0006	MO	64801H	H7-1	300	63.0	67	0.72	NS	
2	74401G	4E0006	MO	64801H	H7-1	400	65.3	67	0.13	NS	
2	74901H	64801H	H7-1	64801H	H7-1	1052	84.1	87	0.74	NS	
2	74906H	64801H	H7-1	64801H	H7-1	470	94.7	87	5.24	*	Few pollinator plants [6]
2	74908H	64801H	H7-1	64801H	H7-1	65	81.5	87	2.67	NS	
2	74922H	64801H	H7-1	64801H	H7-1	2640	83.1	87	1.34	NS	
2	74922H	64801H	H7-1	64801H	H7-1	7508	37.5 [5]	34	0.72	NS	Homozygous plants selected by PCR
3	84507G	2A0011	MO	74901H	H7-1	462	48.7	50	0.07	NS	Homozygous + Heterozygous H7-1
3	80501A	6E0077	MO	74922H	H7-1	460	65.2	67	0.15	NS	Homozygous + Heterozygous H7-1
3	80502A	6A3971	MO	74922H	H7-1	461	61.6	67	1.32	NS	Homozygous + Heterozygous H7-1
3	80503A	6J0006	MO	74922H	H7-1	460	65.7	67	0.08	NS	Homozygous + Heterozygous H7-1
3	80504A	5J9101	MO	74922H	H7-1	459	66.4	67	0.02	NS	Homozygous + Heterozygous H7-1
3	80506A	7A3774	MO	74922H	H7-1	459	59.1	67	2.82	NS	Homozygous + Heterozygous H7-1
3	80507A	6A2695	MO	74922H	H7-1	449	54.1	67	7.53	*	Homozygous + Heterozygous H7-1 Timing of pollination[6]

CBI-Deleted

Table V-2 (continued. Segregation pattern with Roundup Ready sugar beet event H7-1)

Generation	Identifier	Pedigree[1]				No. plants	% Tolerant plants[3]		Chi-square test		Comment
		Female[2]		Male			Observed	Expected	Chi test value	Sig.[4]	
4	94901H	74922H	H7-1	74922H	H7-1	616	76.3[5]	75	0.09	NS	After selection of heterozygous plants
4	90106J	6J0006	MO	74922H	H7-1	616	53.1	50	0.38	NS	
4	90107J	8J0029	MO	74922H	H7-1	616	52.6	50	0.27	NS	
4	90539A	6A3973	MO	74922H	H7-1	616	55.2	50	1.08	NS	
4	90540A	8A3871	MO	74922H	H7-1	616	52.1	50	0.18	NS	
4	90541A	8J0023	MO	74922H	H7-1	616	51.3	50	0.07	NS	
4	90542A	5J9101	MO	74922H	H7-1	616	54.9	50	0.95	NS	
4	90543A	6J0042	MO	74922H	H7-1	616	47.7	50	0.21	NS	
4	90544A	6E0076	MO	74922H	H7-1	616	46.4	50	0.51	NS	
4	90545A	7J0047	MO	74922H	H7-1	616	49.5	50	0.01	NS	

Legend:

1 See Figure III-2.

2 MO: Monogerm sugar beet material.

3 Selection of plants was made with an application of a Roundup agricultural herbicide at a rate of 12 l/ha in a greenhouse. Homozygous and heterozygous Roundup Ready plants were selected, except as noted in [5].

4 NS: statistically not significant; *: significant at a probability level p=0.05

5 Selection of homozygous/heterozygous plants with PCR markers.

6 These deviations from the expected segregation ratio are likely due either to the small tested population size, the small number of event H7-1 pollinator plants used for seed production, or documented non-optimal timing of pollination during seed production.

CBI-Deleted

Likewise, in a second generational breeding experiment (74906H), there was statistical significance in the segregation data, which was likely due to only a few pollinator plants involved in making the cross. Finally, in a third generational experiment (80507A) the significance in the Chi-square analysis was attributed to non-optimal timing of the pollinator plants used in breeding. Despite these three significant Chi-square values, there are no trends across generations and the Chi-square analysis does indicate that the single T-DNA insert in event H7-1 is integrated in the plant nuclear genome and is inherited as a single locus, following a Mendelian one-locus model, in a stable manner through subsequent generations. These results are consistent with the genetic analyses described in Section V.

B.2. Generation stability: Southern blot analysis

To confirm that the DNA insert in event H7-1 is integrated into the plant genome as a stable insert, Southern hybridizations were performed on genomic DNA extracted from event H7-1 and its progenies over three generations. The original transformation event H7-1 (6401VH) was compared to three progenies (64801H, 74922H and 83002S; see Table III-1 and Figure III-2) of this line resulting from self pollination or crosses with conventional sugar beet lines.

As controls, four different nontransgenic sugar beet lines were analyzed: 3S0057, 5R7150, 8K1180 and 6S0085, where 5K7150 was control 1, 8K1180 was control 2, 6S0085 was control 3 and 3S0057 was control 4 in Figure V-11. Each line's DNA was digested with *Xba*I, *Hind*III and *Bam*HI, respectively, and hybridized with a labeled *cp4 epsps* fragment. If the T-DNA is stably integrated in the plant genome, all progeny containing event H7-1 that are digested with the same restriction enzyme should show a band of the exact same size.

The DNAs from the progenies containing event H7-1 (Figure V-11, lanes 3 to 6) produced the expected fragments: DNA digested with *Bam*HI resulted in a band of approximately 11.0 kb, digestion with *Xba*I produced a fragment of 4.0 kb and HindIII digestion produced a band of 5.2 kb. All hybridization bands from the same digestion, but from different progeny, were identical in molecular weight and mobility, demonstrating that the T-DNA is stably integrated into the plant genome. None of the control lines produced a signal (lanes 1, 2, 7 and 8).

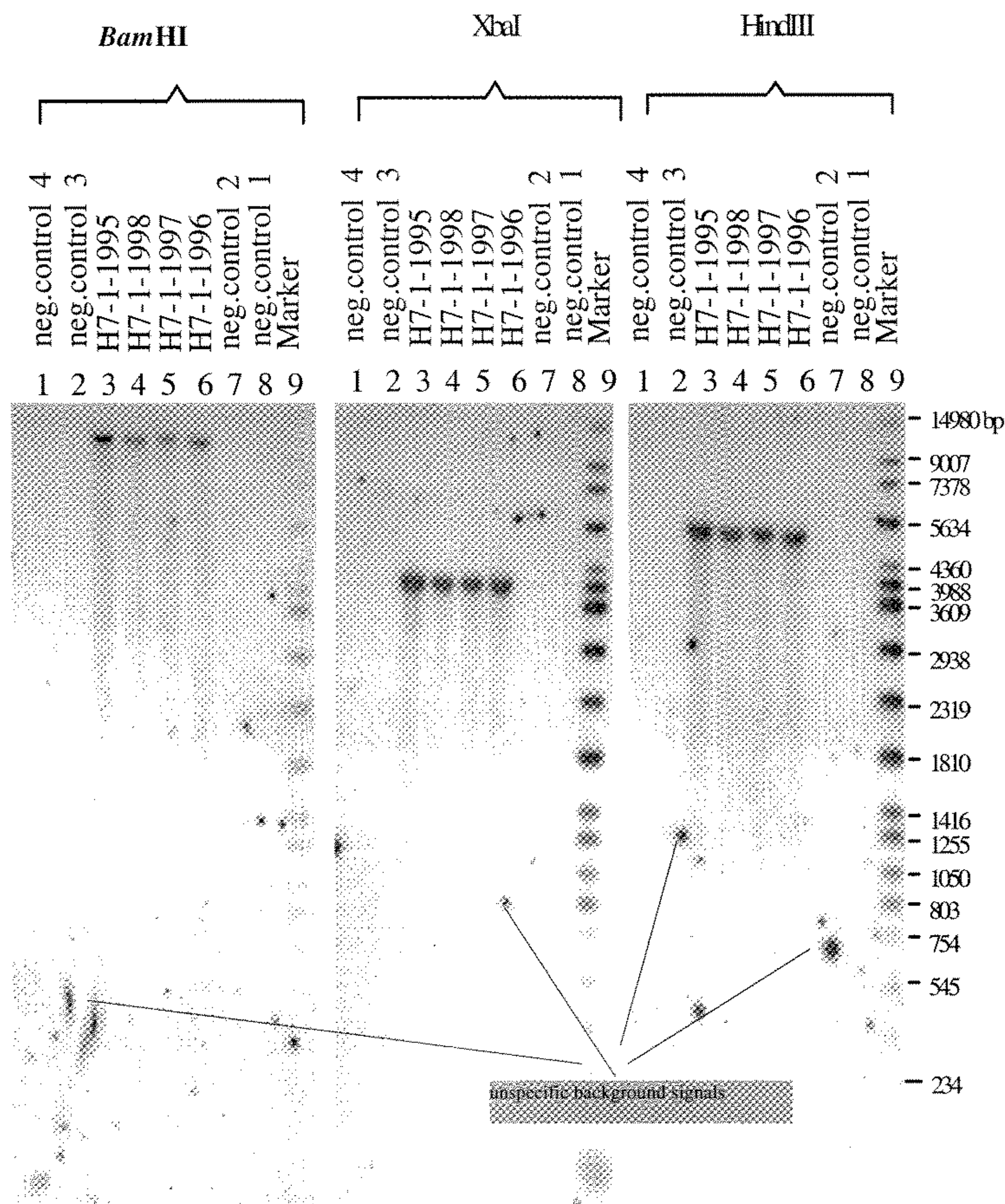
No significant differences in the banding pattern were observed between DNA extracted across multiple generations of event H7-1. These results demonstrate the stability of the inserted DNA across three generations.

B.3. Conclusions

All the observations made for event H7-1, including Southern blot analyses and segregation data based on phenotypic observations, are consistent and demonstrate that the DNA is inherited as a single locus following a Mendelian one-locus model. The

molecular stability of the insert by Southern blot has been demonstrated through three generations.

Figure V-11. Southern blot analysis of event H7-1: Stability analysis



Legend: 10 µg of various event H7-1 genomic DNAs [the original transformant 6401VH (H7-1 – 1995) and three progenies, 64801H (H7-1 – 1996), 74922H (H7-1 – 1997) and 83002S (H7-1 – 1998)] as well as control DNAs from different origins were digested with *Bam*HI, *Xba*I and *Hind*III. The blot was probed with ^{32}P-labeled *cp4 epsps*-PCR probe of PV- BVGT08 (equal to bp 447-1555).

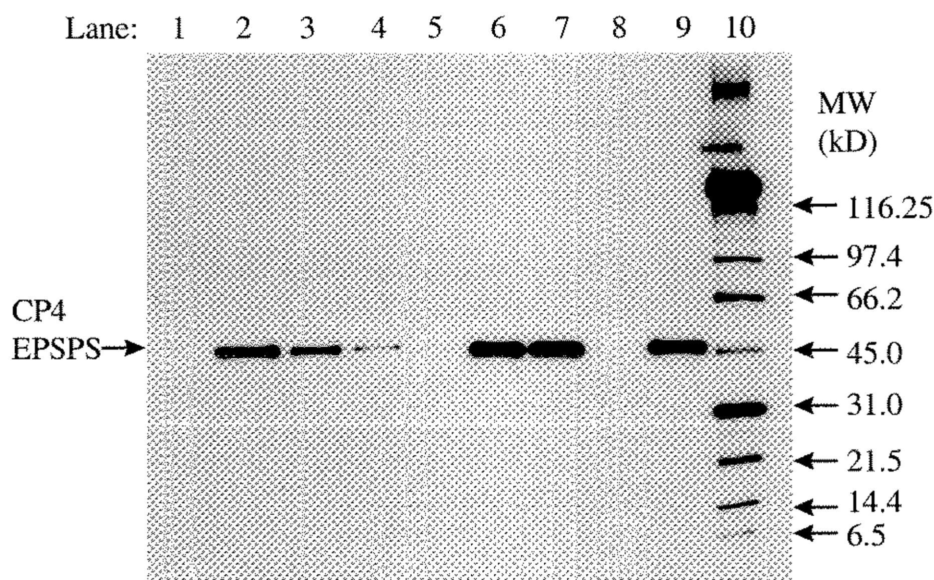
C. Expression of the Inserted *cp4 epsps* Gene

C.1. Characterization of the CP4 EPSPS protein in event H7-1

A western blot analysis, using published methods (Harrison et al., 1996), was conducted to assess the equivalence of the CP4 EPSPS protein produced in event H7-1 to the CP4 EPSPS protein produced in *E. coli*, as well as to that produced in commercial Roundup Ready soybeans.

This western blot (Figure V-12) shows that only one immuno-reactive protein of the expected apparent molecular weight is found in extracts of event H7-1. These results demonstrate that the CP4 EPSPS protein in event H7-1 is equivalent to those produced in *E. coli* and in commercial Roundup Ready soybeans. This supports the use of the *E. coli*-produced CP4 EPSPS protein as a reference standard in the ELISA assay used to estimate the levels of the CP4 EPSPS protein in event H7-1, as described in the following section.

Figure V-12. Western blot of CP4 EPSPS protein produced by *E. coli*, Roundup Ready soybean and event H7-1



Legend:
Lane 1: Amersham full range color markers (not observable in this format).
Lane 2: 2 ng CP4 EPSPS standard spiked into control sugar beet matrix.
Lane 3: 1 ng CP4 EPSPS standard spiked into control sugar beet matrix.
Lane 4: 0.5 ng CP4 EPSPS standard spiked into control sugar beet matrix.
Lane 5: Control sugar beet extract.
Lane 6: Event H7-1 extract.
Lane 7: Roundup Ready soybean (event 40-3-2) soybean extract.
Lane 8: Control soybean extract.
Lane 9: 2 ng of CP4 EPSPS standard spiked into control (line A3244) soybean matrix.
Lane 10: BioRad Biotinylated MW markers, Cat. No. 161-0319.

C.2. Levels of CP4 EPSPS protein in event H7-1

CP4 EPSPS protein levels in sample extracts of leaf (top) and processed root (brei) tissues were estimated using a double-antibody sandwich ELISA, consisting of a mouse monoclonal anti-CP4 EPSPS antibody as the capture antibody and a goat polyclonal anti-CP4 EPSPS conjugated to horseradish peroxidase (HRP) as the detection antibody. A horseradish peroxidase substrate, TMB (3,3',5,5' tetramethylbenzidene), was added for color development. The CP4 EPSPS protein levels in plant tissue extracts were estimated by comparison of the sample absorbance (OD) to the absorbance produced by a range of concentrations of the *E. coli*-produced CP4 EPSPS reference standard. The CP4 EPSPS protein standard was purified from an *E. coli* strain expressing the *Agrobacterium* sp. strain *cp4 epsps* gene. The protein standard has been previously characterized (Harrison et al., 1996).

In 1999, field trials were conducted at six distinct field locations distributed across Europe in the major sugar beet production areas. The event H7-1 sugar beets were treated with a Roundup agricultural herbicide. Samples of brei (root tissue processed using standard sugar beet industry methods) and top (leaf) tissues were collected and analyzed for levels of the CP4 EPSPS protein using ELISA. On average, concentrations of the CP4 EPSPS protein, on a fresh weight basis, were similar in the leaf tissue (161 μg/g) and in the root tissue (181 μg/g). The range of mean levels of the CP4 EPSPS protein in top (leaf) tissue were 112 to 201 μg/g and in root (brei) were 145 to 202 μg/g across the sites. Results are summarized in Table V-3.

Table V-3. Summary of CP4 EPSPS levels in tissues of event H7-1

Tissue Type	CP4 EPSPS Protein (µg/g tissue fresh weight)
Top[1]	
mean[2]	161
range[3]	112 to 201
Brei[4]	
mean[2]	181
range[3]	145 to 202

Legend:

[1] One leaf approximately 5-10 cm² was sampled from 30 event H7-1 plants for each replicate. Three replicates per site were collected. Collected leaves were placed in conical tubes and transferred on dry ice to the testing facility.

[2] The mean was calculated from the analyses of three replicate plant samples, from each of the field sites.

[3] Range of mean values from the analyses of samples at each site. Where in top (leaf) n = 6 sites and in root (brei) n = 6 sites.

[4] Brei was prepared by a French laboratory, AGREN, using a sawing machine. Samples were immediately frozen on dry ice and then stored in a –80°C freezer until analysis.

C.3. Conclusions

The mean levels of CP4 EPSPS protein present in event H7-1 top and root tissues were similar. No meaningful differences in the range of CP4 EPSPS protein mean levels were observed across sites for the event H7-1 tissues.

D. CP4 EPSPS Protein Specificity and Homology to EPSPSs Derived from a Variety of Plant and Microbial Sources

The *cp4 epsps* gene was obtained from the naturally occurring, glyphosate-degrading bacterium identified as *Agrobacterium* sp. strain CP4 (Padgette et al., 1996). The gene encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids. CP4 EPSPS protein catalyzes a non-rate limiting step in the shikimate pathway involved in aromatic amino acid biosynthesis in bacteria and plants. The enzymatic activity of EPSPSs from a variety of glyphosate-tolerant and sensitive plant and microbial sources has been extensively characterized. It has been established that the CP4 EPSPS protein is highly specific for its natural substrates, shikimate-3-phosphate and phosphoenolpyruvate (Padgette et al., 1995, 1996; Franz et al., 1997). This characterization included an examination of the three-dimensional folding patterns and active site homology. The EPSPS derived from *Agrobacterium* sp. strain CP4 is structurally and functionally similar to native EPSPSs in plants and microbes that are commonly consumed by humans, but retains its catalytic activity in the presence of the inhibitor, glyphosate (Padgette et al., 1995). The shikimate pathway is not present in mammals, which contributes to the very favorable toxicological profile for glyphosate (Williams et al., 2000).

EPSPS is ubiquitous in plants and microbes, and as such, this class of proteins are not associated with plant pest properties. The CP4 EPSPS protein shares sequence homology with EPSPS naturally present in plants, as well as in ubiquitous fungal and microbial sources such as *E. coli*, *Saccharomyces cerevisiae* (baker's yeast) and *Bacillus subtilis* (Mountain, 1989), as shown in Table V-4 (Padgette et al., 1996).

Table V-4. Comparison of the deduced amino acid sequence of native CP4 EPSPS to that of other EPSPSs

	soybean	corn	*E. coli*	*B. subtilis*	*S. cerevisiae*	*Roundup Ready crops**
CP4 EPSPS						
% sequence identity	26	24	26	41	30	>99
% sequence similarity	51	49	52	59	54	>99

Legend:
* Roundup Ready soybean, NK603 corn, canola, cotton and sugar beet event 77.

The ubiquitous presence of homologous EPSPS enzymes in food crops and common microbes establishes that EPSPS proteins, and their enzyme activity, pose no hazards for human consumption or to the environment. Event H7-1, containing the CP4 EPSPS protein, is considered not to be significantly different than crops containing their native EPSPS and no different than other CP4 EPSPS protein-containing crops previously reviewed and granted nonregulated status, including Roundup Ready soybean, NK603 corn, canola, sugar beet event 77 and cotton. The following data in Part VI support this conclusion that event H7-1 is not significantly different and is no more likely to pose a plant pest risk than conventional sugar beet.

VI. AGRONOMIC EVALUATION

This section provides an agronomic evaluation of Roundup Ready sugar beet event H7-1, including disease and pest susceptibility assessments from field testing information and USDA-APHIS field reports, agronomic characteristics and crop composition. These data were used to determine that the regulated article, event H7-1, is no more likely to pose a plant pest risk than conventional sugar beet.

A. Disease and Pest Susceptibilities

Disease and pest susceptibility information is used to determine if the genetic modification to produce event H7-1 has altered plant-disease or plant-pest interactions compared to conventional sugar beet. Observations from three sets of trials conducted with event H7-1 support the determination that event H7-1 does not have altered plant-pest risk. These data were obtained from: 1) nursery trials conducted in the U.S. for variety approval; 2) standard industry field trials conducted in the U.S.; and 3) field trials conducted in Europe.

A.1. Nursery trials conducted in the U.S.

During the 2000 and 2001 growing seasons, quantitative data were collected from official nursery trials. The nursery trials are conducted each year to assess new sugar beet candidates being considered for introduction in order to gain variety approval. Roundup Ready sugar beet event H7-1 was included in these replicated nursery trials for comparison to registered, conventional sugar beet varieties for relative resistance against infection from *Cercospora* leaf spot, *Aphanomyces* root rot, curly top and *Rhizoctonia* root rot.

A.1.a. *Cercospora* leaf spot nursery trials. The official *Cercospora* leaf spot nurseries were established in 2000 and 2001 by Betaseed, Inc., a wholly owned subsidiary of KWS. These trials were conducted to gain variety approval from the American Crystal Sugar Company, the Minn-Dak Farmers Cooperative and the Southern Minnesota Beet Sugar Cooperative. The *Cercospora* leaf spot nurseries were artificially inoculated in both years.

In 2000, the official *Cercospora* leaf spot nursery trial was established at a single site with 203 variety candidates entered, including the coded entries Beta 991 RR and Beta 992 RR, which contain event H7-1. In this nursery trial, each entry had six replicate plots established in a randomized complete block design. The plots consisted of two rows planted on 22 inch centers that were 10 feet in length. *Cercospora* leaf spot foliar infection ratings in the Betaseed nurseries were taken six times during the 2000 growing season. *Cercospora* ratings were taken on approximately 180 plants when the disease symptoms first appeared, which normally occurs the last week of July, and continued until plant regrowth begins, usually by the end of August. The rating dates during the 2000 nursery were as follows: 27 July, 2 August, 7 August, 11 August, 21 August and 25

August, with the ratings from 2 August and 7 August averaged to provide a single rating value. The ratings data are summarized in Table VI-1.

Table VI-1. Comparative analysis of disease ratings for *Cercospora* resistance of event H7-1

	Cercospora[1] mean ratings	
Variety Description	**2000[2]**	**2001[3]**
Beta 6447	4.95	4.81
Crystal 222	4.98	NA
Hilleshög Blazer	4.72	5.04
Van der Have H66156	5.24	4.73
Beta 991 RR[4]	5.38	4.61
Beta 992 RR[4]	4.04	NA
Beta 1092 RR[4]	NA	3.97
C.V.[5] (%)	7.0	7.5
LSD[6] (0.05)	0.35	0.39

Legend:
[1] *Cercospora* leaf spot resistance rating using a 1-9 scale, where 1 is excellent, 9 is poor (season means are reported).
[2] n = one nursery site in 2000 (USDA-APHIS notification 00-067-21n)
[3] n = two nursery sites in 2001 (USDA-APHIS notification 01-078-12n)
[4] Beta 991 RR, Beta 992 RR and Beta 1092 RR contain event H7-1.
[5] Coefficient of variation.
[6] Least significant difference used to discern differences at the 0.05 level of significance.
NA = not available

In 2001, two *Cercospora* nursery trials were established. One trial was conducted for the American Crystal Sugar Company, in conjunction with the Minn-Dak Farmer Cooperative, and the second was conducted for the Southern Minnesota Beet Sugar Cooperative. In the American Crystal Sugar Company nursery trial, 182 variety candidates (including the coded entries Beta 991 RR and Beta 1092 RR, which contain event H7-1) were entered. In the Southern Minnesota Beet Sugar Cooperative nursery trial, 64 variety candidates (including the coded entries Beta 991 RR and Beta 1092 RR) were entered into the nursery trial. In both of these 2001 nursery trials, each entry had six replicate plots established in a randomized complete block design. The plots consisted of two rows planted on 22-inch centers that were 10 feet in length. *Cercospora* ratings were taken on approximately 180 plants when the disease symptoms first appeared and continued until plant regrowth began. *Cercospora* leaf spot foliar infection ratings were taken six times during the 2001 growing season. The rating dates were as follows: 24 July, 29 July, 3 August, 11 August, 16 August and 21 August, with the rating values from 29 July and 3 August averaged to provide a single rating value.

The ratings data from the 2000 and 2001 nurseries are summarized in Table VI-1. The ratings values are presented as a mean for each year, which is the standard reporting format for nursery trials. The resistance of Roundup Ready sugar beet event H7-1 (Beta 991 RR, Beta 992 RR and Beta 1092 RR) to *Cercospora* infection across the two growing seasons was shown to either be within the range of ratings observed for conventional registered varieties already in commerce or had lower ratings than the conventional varieties indicating better resistance to *Cercospora* leaf spot, likely due to the event H7-1 candidates' genotype.

Additionally in 2001, an independent *Cercospora* leaf spot nursery trial was established by the Michigan Sugar and Monitor Sugar Companies at a single site in Michigan. This *Cercospora* leaf spot nursery trial had 51 variety candidates entered (including the coded entry Beta 992 RR, which contains event H7-1). In this nursery trial, each entry had five replicate plots established in a randomized complete block design. The plots consisted of two rows planted on 30-inch centers that were 15 to 20 feet in length. *Cercospora* leaf spot foliar infection ratings in this nursery were taken on four dates by the Monitor Sugar Company, and on seven dates by the Michigan Sugar Company. The plot ratings were averaged to obtain a season mean value, as is the accepted reporting format for a nursery trial. The season mean value for Beta 992 RR was 3.0. This low rating, indicating greater resistance, is comparable to the 2000 rating for Beta 992 RR reported in Table VI-1.

A.1.b. *Aphanomyces* root rot nursery trials. The official *Aphanomyces* root rot nurseries, which were established in 2000 and 2001 by Betaseed, Inc., were conducted to gain variety approval from the American Crystal Sugar Company, the Minn-Dak Farmers Cooperative and the Southern Minnesota Beet Sugar Cooperative. The *Aphanomyces* nurseries were established under naturally occurring infection during both years.

In 2000, the official *Aphanomyces* root rot nursery trial was established at a single site with 137 variety candidates entered (including coded entries Beta 991 RR and Beta 992 RR, which contain event H7-1). In this nursery trial, each entry had six replicate plots established in a randomized complete block design. The plots consisted of two rows planted on 22-inch centers that were 10 feet in length. Ratings for the severity of mid-season foliar *Aphanomyces* symptoms were taken once during the 2000 nursery on approximately 360 plants. Foliar ratings are taken when disease symptoms occur among the official check varieties entered in the trial, usually during the first week of July. Ratings for end-of-season severity of *Aphanomyces* root rot are taken when the roots are dug near the end of September. The foliar rating was taken on 7 August and the root rot rating was taken on 3 October.

In 2001, an official *Aphanomyces* nursery trial was established at one site. One series of the trial was conducted for the American Crystal Sugar Company, in conjunction with the Minn-Dak Farmer cooperative, and the second series was conducted for the Southern Minnesota Beet Sugar Cooperative. In the American Crystal Sugar Company nursery trial

series, 114 variety candidates (including coded entries Beta 991 RR and Beta 1092 RR, which contain event H7-1) were entered. In the Southern Minnesota Beet Sugar Cooperative nursery trial series, 64 variety candidates (including coded entries Beta 991 RR and Beta 1092 RR) were entered. In both series of this 2001 nursery trial, each entry had six replicate plots established in a randomized complete block design. The plots consisted of two rows planted on 22-inch centers that were 10 feet in length. Ratings for the severity of mid-season foliar *Aphanomyces* symptoms were taken during the 2001 nursery on approximately 360 plants. The foliar ratings were taken on 18 July and 13 August; with the root rot rating taken on 19 September.

The ratings data from the 2000 and 2001 nurseries are summarized in Table VI-2, where the mean of the two 2001 ratings, which is the accepted reporting format for nursery trials, is reported. The resistance of Roundup Ready sugar beet event H7-1 (Beta 991 RR, Beta 992 RR and Beta 1092 RR) to *Aphanomyces* infection was shown to be within the range of ratings observed for conventional registered varieties already in commerce.

Table VI-2. Comparative analysis of disease ratings for *Aphanomyces* resistance of event H7-1

	***Aphanomyces*[1] foliar mean rating**		***Aphanomyces*[1] root rot mean ratings**	
Variety Description	**2000[2]**	**2001[3]**	**2000[2]**	**2001[3]**
Beta 6447	3.50	3.00	5.50	4.67
Crystal 222	2.83	NA	4.67	NA
Hilleshög Blazer	3.00	4.50	5.00	6.17
Van der Have H66156	4.67	3.92	5.83	5.83
Beta 991 RR[4]	3.30	3.33	5.17	5.67
Beta 992 RR[4]	3.38	NA	5.50	NA
Beta 1092 RR[4]	NA	3.75	NA	5.50
C.V.[5] (%)	18.2	19.9	13.5	14.0
LSD[6] (0.05)	0.75	0.87	0.79	0.89

Legend:
[1] *Aphanomyces* symptom evaluations. Lower numbers indicate greater resistance.
[2] n = one nursery site in 2000 (USDA-APHIS notification 00-067-21n)
[3] n = one nursery site in 2001 (USDA-APHIS notification 01-078-12n)
[4] Beta 991 RR, Beta 992 RR and Beta 1092 RR contain event H7-1.
[5] Coefficient of variation.
[6] Least significant difference used to discern differences at the 0.05 level of significance.
NA = not available

A.1.c. Curly top and *Rhizoctonia* root rot nursery trials. Additional nurseries were established in 2000 and 2001 to assess the relative resistance of sugar beet variety candidates to curly top and *Rhizoctonia* root rot resistance.

In 2000, a curly top nursery was established at one site by Betaseed, Inc. In this nursery trial, each entry had three replicate plots established in a randomized complete block design. The plots consisted of two rows planted on 22-inch centers that were 10 feet in length. Foliar ratings for curly top normally begin in the middle of August, with a total of two to three ratings taken on approximately 180 plants per season. Ratings for the 2000 nursery occurred twice during the growing season on 21 September and 9 October.

The 2001 curly top nursery, established at one site, was an official nursery trial for the Western Sugar Company. In this nursery trial, each entry had four replicate plots established in a randomized complete block design. The plots consisted of two rows planted on 22-inch centers that were 10 feet in length. The rating dates for the 2001 nursery occurred twice over two-day periods. These ratings occurred on 17-18 August and 30-31 August. Ratings for curly top resistance in 2000 and 2001 are reported in Table VI-3.

Table VI-3. Comparative analysis of disease ratings for curly top and *Rhizoctonia* root rot resistance of event H7-1

	2000	2001	
Variety Description	Curly Top Foliar Rating[1]	Curly Top Foliar Rating[1]	*Rhizoctonia* Root Rot Rating[2]
Beta 8757	5.00	5.19	NA
Beta 8754	7.20	6.04	5.40
Beta 4546	NA	5.31	3.00
Beta 4776R	6.10	NA	NA
Beta 8757 RR[3]	5.20	5.77	4.70
Beta 1054 RR[3]	NA	5.95	NA
Beta 1046 RR[3]	NA	NA	3.50
Crystal 9930 RR[3]	NA	4.97	NA

Legend:
[1] The foliar rating scale for curly top is from 1 to 9, with 1 being healthy and 9 being dead.
[2] The rating scale for *Rhizoctonia* root rot is from 0 to 7, with 0 being healthy and 7 being dead.
[3] All Beta RR and Crystal RR varieties contained event H7-1 (USDA-APHIS notifications: year 2000 trials = 00-062-10n and year 2001 trials = 01-054-08n).
NA = not available

The *Rhizoctonia* root rot nursery was established at one site in 2001 by the Beet Sugar Development Foundation, as an official nursery for the Western Sugar Company. In this nursery trial, each entry had five replicate plots established in a randomized completed block design. The plots consisted of a single row that was 12 feet in length, planted on 22-inch centers from other plot rows. In the *Rhizoctonia* root rot nursery, individual roots were dug and rated when the susceptible control varieties entered in the nursery were

killed by *Rhizoctonia*. The rating typically occurs in early September on approximately 95 plants, and the rating is reported as a plot mean. For evaluation purposes, the ratings for event H7-1 were compared to the values observed for several registered conventional varieties. Ratings for *Rhizoctonia* root rot resistance are reported in Table VI-3.

Based on the resistance ratings in Table VI-3, Roundup Ready sugar beet event H7-1 had curly top virus and *Rhizoctonia* root rot ratings that were within the range of or better than, likely due to the event H7-1 candidate's genotype, the ratings for registered conventional sugar beet varieties already in commerce.

A.2. Field trials conducted in the U.S.

The U.S. field trials conducted between 1998 and 2002 were established according to industry standards for sugar beet varietal development. During these standard industry field trials with event H7-1, researchers were requested to monitor field sites for disease and insect susceptibility at least on a monthly basis during the growing season, but assessments typically were performed on a biweekly basis. These disease and insect susceptibility assessments were reported to the USDA-APHIS in the final field reports. Visual observations were made while walking the fields and, in almost all circumstances, the observations were qualitative rather than quantitative. While these assessments are primarily qualitative, the in-field monitoring provides disease and insect susceptibility observations for event H7-1 from differing environments, geographies and growing years, which are used to establish whether there are plant-disease or plant-pest susceptibility trends associated with event H7-1. Completed U.S. standard industry field trials that have been conducted with event H7-1 under USDA-APHIS notification from 1998 through 2002 are listed in Table VI-4. Final field reports have been submitted to USDA-APHIS for field trials conducted in 1998 - 2002, as directed by USDA-APHIS guidance.

The standard trial types included: 1) proprietary performance trials; 2) official yield performance and disease nurseries; 3) agronomic trials; 4) growout trials; 5) steckling production; and 6) seed multiplication trials. These standard industry field trials followed normal agronomic practices, including weed, disease and insect control measures, where appropriate, to ensure that season-long field data were obtained. The field designs for these trials varied, with the proprietary performance, official yield performance, official disease nursery and agronomic trials typically replicated with at least three replicate plots, while the other trials typically consisted of single plots. Likewise, the comparator for event H7-1 in these trials varied according to the purpose of the trial. Provided below is a description of each trial type with the comparator(s) used and a discussion on why the selected comparator(s) is appropriate.

> Proprietary performance trials: These trials were established by a seed company, such as Betaseed Inc., to generate baseline data on yield, disease and pest resistance for newly developed variety candidates. A newly developed variety candidate is tested in a defined sugar beet production geography, where the germplasm is anticipated to be adapted to the region's environment. During sugar

beet variety development, it is not possible to produce strict isolines (see section B.2). Therefore, in the proprietary performance trials with event H7-1 the control was a variety candidate with "nearly-equivalent" genotype characteristics in a few of the trials, but the principle comparators in these trials were the regionally adapted conventional varieties available to growers.

Official yield performance and disease nursery trials: These performance and nursery trials were conducted by sugar beet nursery agencies or variety approval committees, where the newly developed variety candidates are required to yield comparably to check varieties and have certain levels of disease resistance to specific diseases before they can be released as new varieties for sugar beet producers. There were two sets of comparators in these trials testing event H7-1: first, each performance and nursery trial had baseline conventional varieties, or check varieties, chosen by the respective nursery agency or approval committee conducting the trial, and, second, other variety candidates that were entered into the performance or nursery trial by other seed companies.

Agronomic trials: These field trials were conducted to evaluate typical agronomic performance of event H7-1, such as efficacy of herbicide treatments on weeds present in sugar beet production, beet root and sugar yield of the developed variety candidates, and the emergence, vigor and bolting tendency. The comparators in a few of the trials were variety candidates with "nearly-equivalent" genotype characteristics, but the principle comparators were the regionally adapted conventional varieties.

Growout field trials: These trials were conducted to establish the percentage of glyphosate-tolerant plants (i.e., those plants exhibiting tolerance to Roundup agricultural herbicides due to the presence of the *cp4 epsps* gene) in a breeding population and the tendency of the glyphosate-tolerant plants to bolt. There were no direct in-field comparisons to other "nearly-equivalent" sugar beet variety candidates or regionally adapted conventional varieties in these trials, so comparison was made to conventional varieties grown by local sugar beet producers.

Steckling production trials: The steckling trials were conducted prior to seed multiplication. Sugar beet plants that are planted in the fall, to initiate the vernalization requirement for seed production, are referred to as stecklings. Subsequently, stecklings are transplanted in late winter or early spring into production fields for seed multiplication. There were no designated in-field comparators planted in the steckling production fields, due to the nature of these trials. When additional hybrids or breeding lines, which may or may not be genetically similar, were planted in separate steckling fields near the event H7-1 stecklings, they were used as the comparator.

Seed multiplication trials: These trials were dependent on the steckling production, as described above. Depending on the nature of the individual multiplications, there may or may not be comparators included in these trials. When breeder's seed or stock seed is being produced there are no comparators included in the seed multiplication trials, based on the requirement of defined isolation distances between newly developed hybrids to all other sexually compatible *Beta* sp. to minimize the potential of cross pollination. In this situation, comparison of disease and insect susceptibility is made to conventional varieties grown outside of the isolation zone. When the purpose of the multiplication trial was to produce event H7-1 plants for breeding purposes, male-sterile hybrids or breeding lines genetically dissimilar to the event H7-1 plants were included in the trials, in which case they were used as the comparators.

The disease observations are summarized in Table VI-5. The major diseases of economic importance affecting sugar beet production in the U.S. that were observed in the U.S. standard industry field trials are *Cercospora* leaf spot (*Cercospora beticola*), *Rhizoctonia* root rot (*Rhizoctonia solani*), fungal seedling diseases (*Pythium ultimum, P. aphanidermatum, Rhizoctonia solani* and *Aphanomyces cochlioides*), beet curly top virus, *Rhizomania* (beet necrotic yellow vein virus) and powdery mildew (*Erysiphe betae*). Powdery mildew, *Cercospora* leaf spot and curly top virus were the prevalent diseases noted. A total of 98 separate field trials were conducted during the 1998 – 2001 field seasons. At all but six trial sites, there were no differences (Δ) observed between event H7-1 and the comparator(s).

To determine whether there were biologically relevant trends associated with the six differences noted on disease susceptibility, the six were assessed in more detail to determine a likely cause for the observations. For the disease susceptibility difference associated with notification #00-053-20n, an official yield trial, it was noted that the event H7-1 genotype was the likely cause of increased susceptibility to powdery mildew. For the differences associated with notification #00-067-21n, official *Cercospora* nurseries, at the Huron and Saginaw, Michigan sites, it was also noted that the event H7-1 genotype likely was the cause of increased susceptibility to *Cercospora* leaf spot. For the differences associated with notification #01-008-06n, seed production trials, at the three Lane, Oregon sites, event H7-1 displayed approximately 40% to 50% increased powdery mildew resistance in comparison to the local conventional varieties. In all six instances, the differences were attributed to the event H7-1 genotype. Considering that only six out of the 98 trial sites over four growing seasons indicated a difference in disease susceptibility with no trend associated with event H7-1, there is no apparent disease susceptibility difference between event H7-1 and the comparator(s).

Insect and nematode pests of major economic importance affecting sugar beet production in the U.S. monitored during the U.S. field trials were 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*).

The insect and nematode observations made during the U.S. field trials are summarized in Table VI-6. During the 1998 though 2001 field seasons, the prevalent insect observed was the beet leaf hopper. At all 98 field trials sites, there were no differences (Δ) observed between event H7-1 and the comparators.

In summary, only six out of a total of 98 trial sites over four growing seasons indicated a difference in disease susceptibility with no trend associated with event H7-1. In three of the six trial sites with observed differences in disease susceptibility, event H7-1 had increased resistance to powdery mildew compared to conventional varieties, which is in contrast to the increased susceptibility observed at the other three trials sites. This demonstrates that there were no trends in disease susceptibility with event H7-1 when there were observable differences. In assessing insect and nematode susceptibility of event H7-1 to the comparator(s), there were no observed differences in any of the 98 field trials. Therefore, it is concluded from the results reported in Tables VI-5 and VI-6 that there are no biologically meaningful differences in disease or insect susceptibility between event H7-1 and the nearly-equivalent variety candidates or conventional varieties used as comparator(s). The general lack of differences in response to diseases and insects indicates that event H7-1 is unlikely to exhibit enhanced pest potential.

In addition to assessing the susceptibility of event H7-1 to common diseases present in U.S. field trials, an independent greenhouse assessment of *Fusarium* yellows has been conducted with event H7-1. While this assessment was not conducted to support the event H7-1 nonregulated status petition, it does provide supplemental information. Preliminary results appear to indicate there are no susceptibility differences between event H7-1 and its control variety, with "nearly-equivalent" genotype characteristics, when inoculated with three of the four *Fusarium* sp. isolates tested (Hanson, 2003). A susceptibility difference was observed between event H7-1 treated with a Roundup agricultural herbicide and the comparator when inoculated with one *Fusarium* sp. isolate.

The susceptibility difference noted in this greenhouse assessment was not likely due to event H7-1 being treated with glyphosate, based upon currently available scientific information, but was more likely due to artificial laboratory conditions used to obtain the preliminary results. Laboratory studies typically utilize nutrient-rich growth media and bacterial cultures acclimated to artificial laboratory conditions; these conditions have led several researchers to conclude that it is difficult to extrapolate results from the laboratory to the natural soil environment (Estok et al., 1989; Wan et al., 1998). Recently, two other reports have suggested that glyphosate-tolerant crops may be more susceptible to infestation by soil-borne plant pathogens than commercial varieties of the same crop (Kremer et al., 2000; Termorshuizen and Lotz, 2002). The authors hypothesized that the change in pathogen populations and suppressive effects of glyphosate herbicide could lead to increased incidence of diseases such as sudden death syndrome (SDS) and Pythium root rot. However, while these studies demonstrate that some changes in population levels of pathogens in soil can occur, there is no evidence in the field of increased incidence of disease or effects on yield. When comparing disease tolerance

Table VI-4. USDA-APHIS notifications relevant to the field testing of event H7-1

Year	USDA Notification No.	Release Sites Covered by Notification	Trial Type
1998 Field Trials	98-065-06n	ID, MN	Proprietary trials
	98-219-06n	AZ	Proprietary trial
1999 Field Trials	99-110-21n	ND, MN	Agronomic trials
	99-060-10n	ID, ND	Proprietary trials
	99-152-01n	OR	Steckling production
2000 Field Trials	00-034-01n	OR	Seed multiplication
	00-049-09n	ID	Proprietary trial
	00-053-20n	WA	Official yield nursery
	00-061-06n	CA	Official disease nursery
	00-061-09n	ID, MI, ND, NE	Agronomic trials
	00-062-10n	CA, ID, MT, WY, NE, OR, CO	Official yield and disease nurseries
	00-067-19n	MN, ND	Agronomic trial
	00-067-21n	MI, MN, ND	Official yield and disease nurseries
	00-180-06n	CA	Official yield nursery
	00-187-02n	OR	Growout/Steckling
	00-224-05n	AZ	Steckling production
2001 Field Trials	01-008-06n	OR	Seed multiplication
	01-054-08n	OR, ID, CO, CA	Official yield and disease nurseries
	01-054-09n	ID	Proprietary trial
	01-066-01n	WA	Agronomic trial
	01-074-17n	CO, MT, NE, WY	Official yield nurseries
	01-074-19n	NE	Agronomic trial
	01-078-12n	MI, MN, ND	Official yield and disease nurseries
	01-093-10n	MI	Trial canceled
	01-115-02n	ID, MN, OR	Growout trial
	01-192-07n	AZ	Growout trial
	01-219-06n	AZ	Steckling production
	01-250-06n	CA	Trial canceled
2002 Field Trials	02-008-01n	OR	Growout/seed multiplication
	02-057-02n	NE	Agronomic trial
	02-063-12n	MN, ND	Official yield and disease nurseries
	02-063-14n	MN	Official nursery
	02-070-01n	ID, MN, OR	Growout trial
	02-144-10n	OR	Steckling production
	02-210-02n	AZ	Steckling production

Table VI-5. Disease observations for event H7-1 in multiple U.S. field trials

USDA No.	County	State	No. Obs.	PM	CLS	RhR	AP	CT	RZM	Δ
98-219-06n	Pinal	AZ	12							no
99-060-10n	Trail	ND	NA		√		√			no
99-060-10n	Twin Falls	ID	7	√				√	√	no
99-152-01n	Douglas	OR	1							no
00-034-01n	Lane	OR	2							no
00-034-01n	Lane	OR	2							no
00-034-01n	Lane	OR	2							no
00-034-01n	Lane	OR	2							no
00-049-09n	Twin Falls	ID	12	√				√		no
00-049-09n	Canyon	ID	12	√				√		no
00-049-09n	Cassia	ID	12	√				√		no
00-049-09n	Twin Falls	ID	2							no
00-053-20n	Adams	WA	13	√						yes[1]
00-061-06n	Yolo	CA	2							no
00-062-10n	Richland	MT	1							no
00-062-10n	Park	WY	4							no
00-062-10n	Larimer	CO	4							no
00-062-10n	Scotts Bluff	NE	5							no
00-062-10n	Bighorn	MT	4							no
00-062-10n	Monterey	CA	12	√					√	no
00-062-10n	Weld	CO	3			√				no
00-062-10n	Bingham	ID	3							no
00-062-10n	Twin Falls	ID	3							no
00-062-10n	Twin Falls	ID	12					√		no
00-062-10n	Malheur	OR	5							no
00-067-19n	Cass	ND	2							no
00-067-19n	Polk	MN	4							no
00-067-21n	Bay	MI	NA							no
00-067-21n	Bay	MI	NA							no
00-067-21n	Gratiot	MI	NA		√					no
00-067-21n	Saginaw	MI	NA		√					no
00-067-21n	Huron	MI	NA		√					no
00-067-21n	Huron	MI	NA		√					yes[2]
00-067-21n	Saginaw	MI	5		√					yes[2]
00-067-21n	Saginaw	MI	2		√					no
00-067-21n	Dakota	MN	5		√					no

Table VI-5 (continued). Disease observations for event H7-1 in multiple U.S. field trials

USDA No.	County	State	No. Obs.	PM	CLS	RhR	AP	CT	RZM	Δ
00-067-21n	Scott	MN	6		√					no
00-067-21n	Scott	MN	5				√			no
00-067-21n	Clay	MN	4							no
00-067-21n	Polk	MN	4							no
00-067-21n	Grand Forks	ND	5							no
00-067-21n	Pembina	ND	6							no
00-067-21n	Richland	ND	5							no
00-067-21n	Richland	ND	4							no
00-067-21n	Traill	ND	4							no
00-067-21n	Wilkin	MN	5							no
00-067-21n	Chippewa	MN	4							no
00-067-21n	Chippewa	MN	4							no
00-067-21n	Renville	MN	4							no
00-067-21n	Renville	MN	4							no
00-180-06n	Imperial	CA	12	√					√	no
00-187-02n	Douglas	OR	NA							no
00-187-02n	Linn	OR	12							no
00-224-05n	Yuma	AZ	2							no
01-008-06n	Lane	OR	5	√						yes[3]
01-008-06n	Lane	OR	6	√						yes[4]
01-008-06n	Lane	OR	6	√						yes[5]
01-008-06n	Lane	OR	5							no
01-054-09n	Canyon	ID	5	√				√		no
01-054-09n	Cassia	ID	7	√				√		no
01-054-09n	Twin Falls	ID	5	√				√		no
01-054-09n	Power	ID	4							no
01-054-08n	Malheur	OR	5							no
01-054-08n	Twin Falls	ID	6							no
01-054-08n	Twin Falls	ID	2					√		no
01-054-08n	Larimer	CO	4			√				no
01-054-08n	Monterey	CA	2						√	no
01-074-19n	Scotts Bluff	NE	5							no
01-074-17n	Box Butte	NE	9		√	√				no
01-074-17n	Weld	CO	5							no
01-074-17n	Park	WY	5							no
01-074-17n	Bighorn	Mt	5			√				no

Table VI-5 (continued). Disease observations for event H7-1 in multiple U.S. field trials

USDA No.	County	State	No. Obs.	PM	CLS	RhR	AP	CT	RZM	Δ
01-078-12n	Saginaw	MI	4							no
01-078-12n	Saginaw	MI	8							no
01-078-12n	Bay	MI	3							no
01-078-12n	Huron	MI	5		√	√				no
01-078-12n	Gratiot	MI	5		√					no
01-078-12n	Tuscola	MI	3		√					no
01-078-12n	Huron	MI	2		√					no
01-078-12n	Traverse	MN	7							no
01-078-12n	Richland	ND	7							no
01-078-12n	Wilkin	MN	11							no
01-078-12n	Pembina	ND	10							no
01-078-12n	Polk	MN	11							no
01-078-12n	Polk	MN	8							no
01-078-12n	Norman	MN	11							no
01-078-12n	Cass	ND	10							no
01-078-12n	Renville	MN	7							no
01-078-12n	Renville	MN	7							no
01-078-12n	Kandiyohi	MN	6							no
01-078-12n	Chippewa	MN	6							no
01-078-12n	Dakota	MN	1		√					no
01-078-12n	Scott	MN	5				√			no
01-115-02n	Linn	OR	7	√						no
01-115-02n	Clay	MN	3							no
01-115-02n	Twin Falls	ID	6							no
01-192-07n	Yuma	AZ	1							no
01-219-06n	Yuma	AZ	1							no

Legend: No. obs: number of observations per site. N.A.: not available. √: indicates disease pressure observed. Disease abbreviations - PM: Powdery mildew (*Erysiphe betae*); CLS: *Cercospora* leaf spot (*Cercospora beticola)*; RhR: *Rhizoctonia* root rot (*Rhizoctonia solani*); AP: fungal seedling disease including *Pythium, Rhizoctonia,* and *Aphanomyces*; CT: Curly top virus and RZM: *Rhizomania.*
Δ: Difference observed between event H7-1 and comparator plants.

[1] On 8/9/2000, powdery mildew observed was more prevalent on event H7-1 plants (likely due to the genetic background of the event H7-1 plants).

[2] *Cercospora* leaf spot was present in the plots: 10% less tolerance for event H7-1 plants (likely due to the genetic background of the event H7-1 plants).

[3] In this case, event H7-1 plants exhibited up to 50% more powdery mildew resistance compared to conventional varieties (likely due to the genetic background of the event H7-1 plants).

[4,5] In these cases, event H7-1 plants exhibited up to 40% more powdery mildew resistance compared to conventional varieties (likely due to the genetic background of event H7-1 plants).

Table VI-6. Observations on insect and nematode damage for event H7-1 in multiple U.S. field trials

USDA No.	County	State	No. obs.	RA	RM	BLH	SBC	RK	Δ
98-219-06n	Pinal	AZ	12						no
99-060-10n	Trail	ND	NA		√				no
99-060-10n	Twin Falls	ID	7		√	√			no
99-152-01n	Douglas	OR	1						no
00-034-01n	Lane	OR	2						no
00-034-01n	Lane	OR	2						no
00-034-01n	Lane	OR	2						no
00-034-01n	Lane	OR	2						no
00-049-09n	Twin Falls	ID	12			√			no
00-049-09n	Canyon	ID	12			√			no
00-049-09n	Cassia	ID	12			√			no
00-049-09n	Twin Falls	ID	2						no
00-053-20n	Adams	WA	13						no
00-061-06n	Yolo	CA	2						no
00-062-10n	Richland	MT	1						no
00-062-10n	Park	WY	4						no
00-062-10n	Larimer	CO	4						no
00-062-10n	Scotts Bluff	NE	5						no
00-062-10n	Bighorn	MT	4						no
00-062-10n	Monterey	CA	12						no
00-062-10n	Weld	CO	3						no
00-062-10n	Bingham	ID	3						no
00-062-10n	Twin Falls	ID	3						no
00-062-10n	Twin Falls	ID	12			√			no
00-062-10n	Malheur	OR	5						no
00-067-19n	Cass	ND	2						no
00-067-19n	Polk	MN	4						no
00-067-21n	Bay	MI	NA						no
00-067-21n	Bay	MI	NA						no
00-067-21n	Gratiot	MI	NA						no
00-067-21n	Saginaw	MI	NA						no
00-067-21n	Huron	MI	NA						no
00-067-21n	Huron	MI	NA						no
00-067-21n	Saginaw	MI	5						no
00-067-21n	Saginaw	MI	2						no
00-067-21n	Dakota	MN	5						no
00-067-21n	Scott	MN	6						no
00-067-21n	Scott	MN	5						no

Table VI-6 (continued). Observations on insect and nematode damage for event H7-1 in multiple U.S. field trials

USDA No.	County	State	No. obs.	RA	RM	BLH	SBC	RK	Δ
00-067-21n	Clay	MN	4						no
00-067-21n	Polk	MN	4						no
00-067-21n	Grand Forks	ND	5						no
00-067-21n	Pembina	ND	6						no
00-067-21n	Richland	ND	5						no
00-067-21n	Richland	ND	4						no
00-067-21n	Traill	ND	4						no
00-067-21n	Wilkin	MN	5						no
00-067-21n	Chippewa	MN	4						no
00-067-21n	Chippewa	MN	4						no
00-067-21n	Renville	MN	4						no
00-067-21n	Renville	MN	4						no
00-180-06n	Imperial	CA	12						no
00-187-02n	Douglas	OR	NA						no
00-187-02n	Linn	OR	12						no
00-224-05n	Yuma	AZ	2						no
01-008-06n	Lane	OR	5						no
01-008-06n	Lane	OR	6						no
01-008-06n	Lane	OR	6						no
01-008-06n	Lane	OR	5						no
01-054-09n	Canyon	ID	5			√			no
01-054-09n	Cassia	ID	7			√			no
01-054-09n	Twin Falls	ID	5			√			no
01-054-09n	Power	ID	4						no
01-054-08n	Malheur	OR	5						no
01-054-08n	Twin Falls	ID	6						no
01-054-08n	Twin Falls	ID	2			√			no
01-054-08n	Larimer	CO	4						no
01-054-08n	Monterey	CA	2						no
01-074-19n	Scotts Bluff	NE	5						no
01-074-17n	Box Butte	NE	9						no
01-074-17n	Weld	CO	5	√					no
01-074-17n	Park	WY	5						no
01-074-17n	Bighorn	Mt	5						no
01-078-12n	Saginaw	MI	4						no
01-078-12n	Saginaw	MI	8						no
01-078-12n	Bay	MI	3						no
01-078-12n	Huron	MI	5						no

Table VI-6 (continued). Observations on insect and nematode damage for event H7-1 in multiple U.S. field trials

USDA No.	County	State	No. obs.	RA	RM	BLH	SBC	RK	Δ
01-078-12n	Gratiot	MI	5						no
01-078-12n	Tuscola	MI	3						no
01-078-12n	Huron	MI	2						no
01-078-12n	Traverse	MN	7						no
01-078-12n	Richland	ND	7						no
01-078-12n	Wilkin	MN	11						no
01-078-12n	Pembina	ND	10						no
01-078-12n	Polk	MN	11						no
01-078-12n	Polk	MN	8						no
01-078-12n	Norman	MN	11						no
01-078-12n	Cass	ND	10						no
01-078-12n	Renville	MN	7						no
01-078-12n	Renville	MN	7						no
01-078-12n	Kandiyohi	MN	6						no
01-078-12n	Chippewa	MN	6						no
01-078-12n	Dakota	MN	1						no
01-078-12n	Scott	MN	5						no
01-115-02n	Linn	OR	7						no
01-115-02n	Clay	MN	3						no
01-115-02n	Twin Falls	ID	6						no
01-192-07n	Yuma	AZ	1						no
01-219-06n	Yuma	AZ	1						no

Legend: No. obs: number of observations. N.A.: not available. √: indicates pest pressure observed. Insect abbreviations - RA: sugar beet root aphid (*Pemphigus populivenae*); RM: sugar beet root maggot (*Tetanops myopaeformis*); BLH: beet leafhopper (*Circulifer tenellus*); SBC: sugar beet cyst nematode (*Heterodera schachtii*) and RK: root knot nematode (*Meloidogyne arenaria, M. incognita, M. javanica* and *M. hapla*). Δ: Difference observed between event H7-1 and comparator plants.

scores of Asgrow's Roundup Ready soybean varieties it was determined that the distribution of low, medium and high disease-tolerant varieties is essentially the same as that observed for genetically similar conventional soybean varieties (Asgrow, 2002). Thus, differences in susceptibility to pathogens in the field may be related to the innate susceptibility of the cultivars or varieties selected and not the presence of the Roundup Ready trait. Additionally, a significant body of research also exists indicating that there is no evidence that the glyphosate tolerance trait is linked to an increase in susceptibility to diseases (Lee et al., 2000; Lightfoot, 2002; Sanogo et al., 2001; Yang et al., 2002).

Collectively, the available scientific evidence indicates that glyphosate-tolerant crops do not have an increased susceptibility to diseases. This observation has been reinforced through results of grower surveys which indicate that the overall performance in the field of glyphosate-tolerant soybeans, as measured by yield and constant increase in acreage, is equal to or greater than that of conventional varieties.

A.3. Field trials conducted in Europe

As additional support, observations for damage or injury from disease and nematodes were made in Europe during the 1998 and 1999 growing seasons, and are provided as supplemental information to the U.S. field trials. In these field assessments, four in France in 1998 followed by two each in France and Germany in 1999, replicated plots included event H7-1, its control with "nearly equivalent" germplasm genetics and regionally adapted conventional sugar beet varieties Roberta, Josepha, Scarlett, Loretta, Tadjana and Wiebke. In all cases, the trials followed European good agricultural practices for sugar beet husbandry.

In both years, these European trials were monitored for the following diseases and nematodes: powdery mildew (*Erysiphe betae*); *Cercospora* leaf spot (*Cercospora beticola)*; downy mildew (*Peronospora farinose)*; *Ramularia* leaf spot (*Ramularia beticola)*; *Alternaria* leaf spot (*Alternaria tenuis)*; *Rhizoctonia* root rot (*Rhizoctonia solani*); sugar beet rust (*Uromyces betae)*; fungal seedling diseases, including *Aphanomyces, Phoma and Pythium*; and Rhizomania and cyst nematode (*Heterodera schachtii*). All of these diseases and pest are also present in the U.S. sugar beet growing regions.

The occurrence and severity of damage and injury due to diseases and nematodes would be expected to vary across geographies and years based on specific environmental factors (e.g., crop rotation, soil type, moisture, relative humidity and temperature), annual differences in the onset and severity of diseases, nematode population dynamics and germplasm susceptibility to pathogen infection and nematode feeding. In these European field trials, no visible symptom of damage or injury due to the listed diseases or nematode was observed in field trials for either event H7-1, the nearly equivalent control or commercial sugar beet varieties in either growing season. While no observable disease or nematode damage was noted in the European trials (Table VI-7), it is expected that the disease and nematode pests native to these regions were likely present, but did not result in the symptoms of disease or infestations at a sufficient level that they were noted during the visual observations. If event H7-1 were significantly more susceptible than the nearly equivalent control or commercial sugar beet varieties to the diseases and pests that were monitored during the two field seasons, event H7-1 would have likely displayed symptoms from the diseases or nematodes expected to be present over the two growing seasons. These observations suggest that event H7-1 is not significantly more susceptible to disease or nematode damage than its nontransgenic nearly equivalent control or conventional sugar beet varieties, supporting the conclusions established in sections A.1 and A.2.

Table VI-7. Observations on disease and nematode susceptibility for event H7-1 in multiple European field locations, 1998-1999

Year	Country	Location	PM	CLS	PF	RB	AT	RhR	UB	AP	RZM	CN
1998	France	Ebouleau	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1998	France	Faucouzy	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1998	France	Villerseau	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1998	France	Fayel	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1999	Germany	Bennigsen	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1999	Germany	Gerbitz	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1999	France	Ebouleau	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1999	France	Monceau	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Legend: ns: no disease or nematode symptoms. Disease abbreviations - PM: Powdery mildew (*Erysiphe betae*); CLS: *Cercospora* leaf spot (*Cercospora beticola)*; PF: Downy mildew (*Peronospora farinose)*; RB: *Ramularia* leaf spot (*Ramularia beticola)*; AT: *Alternaria* leaf spot (*Alternaria tenuis)*; RhR: *Rhizoctonia* root rot (*Rhizoctonia solani*); UB: Sugar beet rust (*Uromyces betae)*; AP: Fungal seedling disease, including *Aphanomyces, Phoma and Pythium*; and RZM: *Rhizomania*. Nematode abbreviation – CN: Cyst nematodes (*Heterodera schachtii*). Δ - difference observed between Roundup Ready sugar beet event H7-1 and comparator plants.

A.4. Disease and pest susceptibility conclusions

In summary, the information obtained from the annual U.S. nursery trials demonstrated that Roundup Ready sugar beet event H7-1 was comparable to conventional varieties, indicating the Roundup Ready trait has not altered the plant-disease interactions and has not had a negative impact on traditional breeding programs. The field monitoring observations on disease, insect or nematode pests in the U.S. standard industry trials detected no biologically meaningful differences in altered plant-disease or plant-pest interaction between event H7-1 and the nontransgenic nearly-equivalent hybrid or conventional varieties. Additionally, in European trials there was no unique phenotype observed that would indicate event H7-1 has significantly greater pest susceptibility characteristics. Collectively, these conclusions support a determination that the regulated article, event H7-1, has no evidence of altered ecological interactions and is no more likely to pose a plant pest risk than conventional sugar beet.

B. Agronomic Characteristics, Performance and Phenotype of Event H7-1

B.1. Coded trials data summary

Sugar beet varieties marketed within the U.S. must meet industry guidelines and performance criteria prior to full market release. There is some variation in these requirements between geographical areas, but variety candidates generally advance from

first-year experimental trials to full-market release after two to three years of multi-location field trials with favorable results. New varieties must meet industry-established levels of recoverable sugar per acre and per ton to be approved. Disease resistance standards must also be met in certain geographical areas. In most areas, varieties not meeting certain requirements are disqualified from further marketing. Joint committees comprised of growers and processor personnel typically make rules and judgments regarding premarket approvals. Historically, the coded variety trials are designed to give an unbiased evaluation of the agronomic fit and genetic potential of sugar beet varieties under several different environments (Steen, 2000).

Roundup Ready sugar beet event H7-1 was entered in various official coded trials beginning with the 2000 growing season. Field information from the 2000 and 2001 trials established and managed by the American Crystal Sugar Company are shown below (Table VI-8). The official coded trial comparators are described in section A.2 (official yield performance trials). The American Crystal Sugar Company production area is the largest contiguous sugar beet production area in the U.S. and their official trials provide comparative data representing this entire area. The coded trials were established using normal agronomic practices for fertility and crop health management at all locations. All entries were established as six row plots with six replications at each location in 2000, and four replications at each location in 2001. Plots were 40 foot in length and planted on rows with 22-inch centers. Planting dates and harvest dates for the five locations in 2000 and four locations in 2001 are listed below in Table VI-8.

Table VI-8. U.S. sugar beet industry coded trials information, 2000 and 2001

2000 Trials[1]

Location	Planting Date	Harvest Date
Grand Forks, ND	5/20/00	10/06/00
Climax, MN	4/24/00	10/18/00
Hillsboro, ND	4/25/00	10/11/00
Felton, MN	4/26/00	10/22/00
Cavalier, ND	5/04/00	10/09/00

2001 Trials[2]

Location	Planting Date	Harvest Date
Casselton, ND	05/16/01	09/24/01
Ada, MN	05/14/01	10/07/01
Crookston, MN	05/13/01	10/06/01
East Grand Forks, MN	05/18/01	09/30/01

Legend:
[1] 2000 coded trials conducted under USDA-APHIS notification number 00-067-21n.
[2] 2001 coded trials conducted under USDA-APHIS notification number 01-078-12n.

Event H7-1, as coded entries Beta 991 RR and 992 RR, was tested in the 2000 coded trials. In the 2001 trials, Beta 992 RR was replaced by a different event H7-1 entry designated as Beta 1092 RR. Four registered, conventional varieties were also included

as entries to establish a performance baseline across locations. The check varieties were chosen by the American Crystal Sugar Company and were identified as the varieties Beta 6447, Crystal 222, Hilleshög Blazer and Van der Have H66156 in the 2000 trials, and Beta 6447, Crystal 817, Hilleshög Blazer and Seedex Monarch in the 2001 trials.

The center four rows of the event H7-1 plots were sprayed three times and two times with a Roundup agricultural herbicide during the 2000 and 2001 growing seasons, respectively. All applications were made at the rate of 32 fluid ounces per acre. In 2000, the first application was made before thinning at the cotyledon to 4-leaf stage, and the second application was made at the 4- to 8-leaf stage. The final application was made at approximately the 8- to 14-leaf stage. In 2001, the first application was made before thinning at the cotyledon to 4-leaf stage, and the second application was made at approximately the 8- to 14-leaf stage. Throughout the growing season, plant traits of agronomic importance were evaluated. Uniform and vigorous seed germination and plant stand establishment are important early season indicators of variety performance. Emergence counts and vigor ratings are usually taken at the 2-leaf stage of crop development. The number of established plants within a standard section for each plot was recorded. Seedling vigor was scored by the size of the young seedlings as a qualitative rating (1-5), as presented in Table VI-9. These data indicate that event H7-1 had vigor ratings comparable to the conventional varieties.

Sugar beet varieties have been selected for biennial behavior, but are managed as an annual plant for sugar production. "Bolters" are considered problematic by growers because they represent individual plants that have made the transition from vegetative to reproductive development. Varieties more recently developed are generally less susceptible to bolting. The percent of bolting plants present within each plot for each entry was recorded, with the trial means provided in Table VI-9. Event H7-1 had similar bolting ratings compared to the conventional varieties. The Beta 991 RR coded entry had a slightly higher percentage of bolters compared to Beta 1092 RR and the conventional varieties in the 2001 trials, which was not observed in the 2000 field trials. Considering the fact that event H7-1 did not display consistent trends of increased bolting over the entries tested or over the two growing seasons, this single observation was likely due to the background genetics, since a new variety can display differing bolting characteristics over growing seasons based on its vernalization induction requirements.

The center two rows of each plot were harvested at the end of the season for sugar beet yield measured in tons per acre. Beets were then processed to determine recoverable sugar in pounds per ton harvested and then the value for recoverable sugar in pounds per acre was calculated. Data values are summarized in Table VI-9. The recoverable sugar from event H7-1 was comparable to or better than the conventional varieties tested.

Values observed in the field for plant vigor, percent bolters, plant emergence and agronomic performance (yield and recoverable sugar in pounds per acre) across locations and years showed that plant growth, development and performance of event H7-1 were no different phenotypically than conventional, commercial varieties. As expected, event

H7-1 was tolerant to either two or three applications of a Roundup agricultural herbicide in the 2000 and 2001 growing seasons, respectively.

Table VI-9. Comparison of event H7-1 to conventional sugar beet varieties for agronomic characteristics

2000 Trial Data[1]

Variety Description	Vigor 1 to 5	Bolters %	Emergence average	Yield Tons/Ac	Sugar[2] lbs/ton	Sugar[2] lbs/Ac
Beta 6447	1.57	0.00	57.5	24.46	345.41	8398.89
Crystal 222	1.61	0.03	68.1	24.85	324.39	8010.49
Hilleshög Blazer	1.68	0.00	60.9	24.55	331.08	8072.54
Van der Have H66156	1.46	0.03	51.6	26.29	323.78	8445.65
Beta 991 RR[3]	1.39	0.03	53.7	27.59	341.42	9348.33
Beta 992 RR[3]	1.17	0.00	56.6	25.51	325.57	8249.37
C.V.[4]	10.68	NA	10.68	4.99	3.55	5.36
LSD[5] (0.05)	5.1	NA	5.10	0.99	7.32	362.57

2001 Trial Data[1]

Variety Description	Vigor 1 to 5	Bolters %	Emergence Average[6]	Yield Tons/Ac	Sugar[2] lbs/ton	Sugar[2] lbs/Ac
Beta 6447	1.41	0.00	NA	22.72	322.60	7344.44
Crystal 817	1.28	0.00	NA	23.95	324.63	7783.20
Hilleshög Blazer	1.84	0.06	NA	22.39	302.48	6781.31
SX Monarch	1.10	0.00	NA	24.07	302.60	7296.65
Beta 991 RR[3]	1.10	0.19	NA	25.85	316.82	8191.92
Beta 1092 RR[3]	0.98	0.06	NA	23.15	307.69	7126.14
C.V.[4]	27.37	NA	NA	5.19	3.59	5.99
LSD[5] (0.05)	0.37	NA	NA	1.2	10.2	484.46

Legend:

NA denotes "not applicable or not available".

[1] Results from official coded trials established and managed by American Crystal Sugar Company.

[2] Recoverable sugar expressed as pounds per ton of beets or pounds per acre.

[3] Beta 991 RR, Beta 992 RR and 1092 RR contain event H7-1.

[4] Coefficient of variation.

[5] Least significant difference used to discern differences at the 0.05 level of significance.

[6] Emergence counts were not conducted on these trials in 2001.

B.2. Plant phenotype of event H7-1

Plant morphological, developmental and inflorescence characteristics of event H7-1 were compared to those of the conventional control line and several KWS breeding lines to determine whether any unintended changes in plant characteristics had occurred as a result of the transformation process or the production of the CP4 EPSPS protein.

In the production of traditionally bred sugar beet hybrids, the breeding process usually involves monocarp, diploid, cytoplasmic male-sterile female lines crossed with a multicarp, diploid or autotetraploid pollinator line. Typically, the diploid pollinator lines are partially inbred lines of the second or third inbred generation. In contrast to other crops, e.g., corn, inbred sugar beet lines are not 100% homozygous. Therefore, within the sugar beet diploid lines there remains some genetic variability, which can be used for further sub-line development. Thus, strict isolines do not exist in sugar beet breeding, in contrast with other hybrid crops such as corn.

Based on the process used to generate the event H7-1 breeding lines and the control line included in this plant phenotypic evaluation, some differences between event H7-1 and the comparator can be anticipated. The control sugar beet line, designated 3S0057, used in the transformation process to produce event H7-1 was a sugar beet pollinator line of the third inbred generation. The resulting event H7-1 plant was multiplied by *in vitro* techniques to produce breeding line 6401VH (see Figure III-2, Part II) under isolated conditions. The seed harvested from line 6401VH has been used to produce pollinator plants containing event H7-1 to derive all subsequent breeding lines. Therefore, all breeding lines containing event H7-1 are sublines of the diploid pollinator line with the inherent genetic variability associated with a diploid line. This genetic variability from the diploid lines can lead to minor differences in agronomic traits relative to the transformant control line 3S0057. However, these differences are expected to fall within the normal range of variation observed by breeders using conventional breeding practices (Borchardt, 1995). For this reason, KWS' standard mulitcarp and monocarp breeding lines were included in the plant phenotype comparisons to provide additional controls for the expected range of genotypic variability in the event H7-1 breeding lines.

Twenty single plants of each genotype (event H7-1; 3S0057, the parental control line; 12 multicarp lines; and 12 monocarp lines) were vernalized, grown and monitored under greenhouse conditions in Einbeck, Germany. Observations were made on hypocotyl color, leaf size (length/width ratio) and the incidence of whole plant chlorosis (chlorophyll defects). Results are summarized in Table VI-10. No differences were observed between event H7-1 and the control, except for a small difference in leaf size with no likely biological significance considering the value fell within the range of values observed for the conventional breeding lines. These data demonstrate that the insertion of event H7-1 has had no effect on the morphological characteristics measured and event H7-1 has no biologically meaningful difference in these characteristics compared to the conventional control.

Table VI-10. Comparison of event H7-1 to conventional control and breeding lines for morphological characteristics

Plant Characteristic[1]	Event H7-1	Conventional Breeding Lines		
		Control Line	12 Multicarp Lines	12 Monocarp Lines
Hypocotyl color [2]	100 % green	100 % green	green – red	green – red
Leaf color [3]	dark green	dark green	pale – dark green	pale – dark green
Leaf chlorosis	not observed	not observed	not observed	not observed
Leaf size (length/width)[4]	1.82	1.92	1.76 – 2.18	1.54 – 2.16

Legend:
[1] Observations or measurements made on 20 greenhouse plants for each genotype.
[2] Hypocotyl color observed and recorded with plants at 2-leaf stage (green or red).
[3] Leaf color was recorded at the 6- to 8-leaf stage (pale, light green, green, dark green).
[4] Plant leaf size measured at the 6- to 8-leaf stage (largest width, leaf length).

In a separate assessment, additional plant growth and developmental characteristics of event H7-1, the control, and other conventional breeding lines were monitored or measured. Seed of the control line, various conventional breeding lines and event H7-1 were produced from various sources, including Einbeck and Kleinwanzleben, Germany greenhouses, or from field trials conducted in either Italy or the U.S. Using vernalized plant material grown from the aforementioned seed materials, greenhouse observations and measurements of plant stem branching (ramification), dates of inflorescence emergence (bolting), the onset of flowering and seed harvest dates were recorded.

The results are summarized in Table VI-11 and the following describes some of the measurements and observations recorded. The ramification type (branching pattern) of five seed-producing plants for each line was recorded. The bolting date was recorded when 50% of the plants showed an elongation of the main tiller greater than 10 cm. The onset of flowering was recorded when 50% of the plants showed open flowers on the secondary branches. An overall rating of the development (classification of plant development) was performed using the flowering and harvest dates to discriminate between six classes (1, very early, to 6, very late). A calculation from the average weight of two subsamples of 200 kernels was recorded and extrapolated to the thousand kernel weight (TKW) of the seed. The seed germination rate of harvested material was measured in the KWS seed quality laboratory.

Some differences were observed between event H7-1 and the control, which is not unexpected given the earlier discussion on genotypic differences inherent in sugar beet breeding lines. The wide variation seen in plant branching for the breeding lines used in hybrid seed production indicates a strong influence of the various genotypes. Differences also were observed in the seed germination rates between event H7-1, the control and the conventional breeding lines, which is likely due to the varying environmental conditions associated with the seed production. The control seed was produced in a 1993 field trial

in Italy under optimal growing conditions, whereas the event H7-1 seed was produced under greenhouse conditions in 1997 and 1998, and the conventional breeding lines were produced under either greenhouse or field conditions, with differing soils and climatic environments, between 1992 and 1998. A difference was also seen in the TKW values for event H7-1 when compared directly to the control. This was also most likely a result of the strong impact of climatic and soil conditions during the seed development and ripening phase in the different environments (e.g., seed production in Italy and U.S. field trials, compared to greenhouses in Germany). Differences in the developmental and ripening phase could have a strong impact on both the development of the seedbearer plants and on seed setting and filling, which could account for the differences observed in the TKW. The observed values for event H7-1 in Table VI-11 were either comparable to the control or fell within the range observed for KWS' conventional multicarp and monocarp breeding lines.

Table VI-11. Comparison of event H7-1 to conventional control and breeding lines for inflorescence and flowering characteristics

		Conventional Breeding Lines		
Plant Characteristic	**Event H7-1**	**Control Line**	**10-12 Multicarp Lines**	**10-12 Monocarp Lines**
Ramification Type	1 dominant main tiller, 20–30 branches of second order, 1–5 branches of third order per each secondary branch	1 dominant main tiller, 25-35 branches of second order, 1-5 branches of third order per each secondary branch	1 dominant main tiller, 12-60 branches of second order, 1-10 branches of third order per each secondary branch	1 dominant main tiller, 8-74 branches of second order, 1-10 branches of third order per each secondary branch
TKW (g)	14.8 – 17.4	20 – 26	14 – 28	8 – 15
Seed germination rate (%)	50 – 95	95	45 – 95	35 – 90
Seed dormancy[1]	not observed	not observed	not observed	not observed
Time for vernalization	12 weeks	12 weeks	12 weeks	12 weeks
Bolting date	72 days after planting	70 days after planting	63 – 72 days after planting	61 – 68 days after planting
Onset of flowering	98 days after planting	98 days after planting	86 – 106 days after planting	80 – 100 days after planting
Seed harvest date	156 days after planting	156 days after planting	141 – 156 days after planting	141 – 156 days after planting
Classification of plant development[2]	5	5	1 – 6	1 – 5

Legend:

[1] During post-release monitoring of seed production field sites, it was observed that viable seeds germinated in the first or second year of monitoring, which is consistent with the expected dormancy of seed from different production years and environments.

[2] Six levels of classification (1- very early to 6- very late).

In summary, 13 morphological and developmental characteristics of event H7-1 were compared to the control and to conventional multicarp and monocarp breeding lines to assess whether unintended changes in plant characteristics may have occurred as a result of the transformation process or the production of the CP4 EPSPS protein. Based on these comparisons, event H7-1 is considered equivalent to conventional sugar beet. These comparisons also demonstrate that the event H7-1 insert and associated phenotype have had no negative effect on the performance of Roundup Ready sugar beets produced through traditional breeding methods. Furthermore, they confirm that event H7-1 bred into conventional sugar beet varieties is similar to traditionally bred sugar beet varieties.

B.3. Agronomic characteristics, performance and phenotype conclusions

Based on an evaluation of the agronomic and phenotypic traits observed, the information included in this section demonstrates that event H7-1 is not meaningfully different in agronomic performance to conventional sugar beet varieties and does not have altered weediness potential. The lack of meaningful differences and altered weediness potential supports the conclusion that Roundup Ready sugar beet event H7-1 is no more likely to pose a plant pesk risk than conventional sugar beet.

C. Sugar Beet Compositional and Quality Component Analyses

Compositional analyses have been conducted on root (brei) and top (leaf) tissue samples obtained from five field sites across Europe in 1999 to assess the compositional equivalence of these components for event H7-1 compared to its control and conventional varieties. All sites were selected as being typical of commercial growing conditions for sugar beets. At each field site, individual plots were established with test, control and commercial reference varieties. The test material was event H7-1 and the control material was the near isogenic null segregant line of event H7-1, with a genetic background similar to event H7-1. The event H7-1 sugar beets were treated with a Roundup agricultural herbicide. At harvest, 30 event H7-1 plants and 25-30 control and conventional reference variety plants were collected from each site's respective test, control and reference plots. Three replicate subplot samples from each of the test, control and reference plots were collected at each site. For each harvested sugar beet sample, the roots were separated from the top (leaf) in preparation for compositional analysis. Root samples were processed into brei prior to analysis.

These analyses included proximate values, carbohydrates, quality parameters, saponins, and eighteen amino acids. The proximate values were analytically determined for root and top samples, which included crude ash, crude fiber, crude fat, crude protein and dry matter. Carbohydrates were determined by calculation. Quality parameters measured in root samples included polarization (percent sucrose), invert sugar, sodium, potassium and alpha-amino nitrogen. All analyses were conducted as a single analysis for the root (brei) and top (leaf) samples from each of the replicated subplot samples.

All compositional analyses were conducted by a contract lab (AGREN, France) using established analytical methods developed for the sugar industry in France (Syndicate National des Fabricants de Sucre).

Compositional data values for event H7-1 were compared to the null segregant control line with a genetic background similar to event H7-1, as well as to commercially available varieties (Access, Opus, Madison, Tatiana, Orbis, Gabriela, Dorotea and Puma) grown in the same field trials. Statistical analyses were conducted by Monsanto on the compositional data combined across sites to identify statistically significant differences between event H7-1 and its control line at $p<0.05$. The compositional analysis results for event H7-1 and the control line are reported in Tables VI-12 through VI-18; the values obtained from the commercial varieties analyzed concurrently and available literature values are provided for comparison.

C.1. Proximate and carbohydrate analyses

Proximate analyses, including dry matter, crude protein, crude fiber, crude ash and crude fat in top and root tissues, were conducted using standard published methods. Overall, there were no statistically significant differences between mean proximate levels in top and root tissues of event H7-1 when compared to the control samples, except for the dry matter mean level in top tissue (Tables VI-12 and VI-13). However, the dry matter mean level and ranges measured in top samples of event H7-1 significantly overlapped the ranges observed for the control, the commercial reference varieties and published values from commercial sugar beet varieties.

Table VI-12. Summary of proximate analyses of top (leaf) tissue from event H7-1

		Control[1]		Event H7-1[1]		Reference Varieties[2]		Literature
Analysis	Unit	Mean	Range	Mean	Range	Mean	Range	Range[3]
Dry matter[4]	%	16.37	13.69-19.83	17.98*	13.94-21.23	16.18	11.37-26.81	16-20
Crude protein[5]	% DM	16.02	14.46-19.80	15.27	11.16-18.31	16.65	12.75-24.47	8.4-23.2
Crude fiber[6]	% DM	11.43	9.23-16.82	11.50	8.56-15.99	11.62	7.84-19.71	5.9-15.9
Crude ash[7]	% DM	19.50	15.39-21.96	21.95	17.84-31.90	21.80	16.20-27.91	11.5-34.4
Crude fat[8]	% DM	0.87	0.74-1.15	0.95	0.85-1.09	1.02	0.53-1.46	0-4.7

Legend:
[1] n = five sites, single analyses
[2] n = five sites, single analyses, eight commercial varieties
[3] Reference DLG 1991.
[4] Dry matter was determined using an oven method.
[5] Crude protein was determined using a Kjeldahl method.
[6] Crude fiber was determined using the Weende analysis.
[7] Crude ash was determined using an oven method.
[8] Crude fat was determined using a Soxhlet method.
* Indicates a statistically significant difference at the 5% level when compared with the corresponding control.

Table VI-13. Summary of proximate analyses of root (brei) tissue from event H7-1

Analysis	Unit	Control[1]		Event H7-1[1]		Reference Varieties[2]		Literature
		Mean	Range	Mean	Range	Mean	Range	Range[3]
Dry matter[4]	%	24.01	21.75-25.83	25.46	22.87-28.88	22.74	19.05-26.33	23
Crude protein[5]	% DM	5.62	4.13-6.98	5.51	4.50-6.57	5.06	3.72-6.93	1.2-12.4
Crude fiber[6]	% DM	4.84	4.57-5.04	4.54	3.73-5.20	4.75	3.65-5.69	3.4-7.4
Crude ash[7]	% DM	2.54	1.78-3.21	2.51	1.73-3.35	2.41	1.74-3.89	1.3-17.7
Crude fat[8]	% DM	0.20	0.06-0.38	0.13	0.08-0.18	0.16	0.05-0.25	0-1.8

Legend:
[1] n = five sites, single analyses
[2] n = five sites, single analyses, eight commercial varieties
[3] Reference DLG 1991.
[4] Dry matter was determined using an oven method.
[5] Crude protein was determined using a Kjeldahl method.
[6] Crude fiber was determined using the Weende analysis.
[7] Crude ash was determined using an oven method.
[8] Crude fat was determined using a Soxhlet method.

Additionally, levels of carbohydrates in the top and root tissue samples of event H7-1 and the control line were determined by calculation and statistically compared. There were no statistically significant differences between mean levels of carbohydrates in top and root samples of event H7-1 when compared to the control. A summary of these data is provided in Table VI-14.

Table VI-14. Summary of carbohydrate determination from event H7-1

Analysis[1]	Unit	Control[2]		Event H7-1[2]		Reference Varieties[3]		Literature
		Mean	Range	Mean	Range	Mean	Range	Range[4]
Top	% DM	52.18	45.50-58.03	50.35	44.60-54.68	48.92	43.30-56.52	38.3-64.5
Root	% DM	86.80	84.44-89.02	87.31	85.58-89.04	87.62	83.31-90.05	67.3-90.9

Legend:
[1] Carbohydrate calculation = 100% - (crude protein + crude ash + crude fibre + crude fat)
[2] n = five sites, single analyses
[3] n = five sites, single analyses, eight commercial varieties
[4] Reference DLG 1991.

C.2. Nutrient quality analyses

Quality analyses of brei samples for sugar content (measured by polarization), invert sugar (glucose + fructose), sodium, potassium and alpha-amino nitrogen were conducted with established procedures used by the sugar industry. The results of the quality analysis of root samples from event H7-1, the control and conventional reference varieties are presented in Table VI-15. The event H7-1 mean levels for the five components measured were not significantly different than the corresponding mean levels in the control.

Table VI-15. Summary of quality analyses of root tissue from event H7-1

		Control[1]		Event H7-1[1]		Reference Varieties[2]		Literature
Analysis	Unit	Mean	Range	Mean	Range	Mean	Range	Range[3]
Polarisation[4]	g/100g FW	18.12	16.11-19.23	18.54	16.14-20.21	17.08	14.14-19.51	10.8-20.7
Potassium[5]	mmol/100g FW	3.85	3.08-4.87	3.85	3.08-5.13	4.10	2.82-5.38	2.95-10.21
Sodium[6]	mmol/100g FW	0.65	0.26-1.83	0.57	0.16-2.04	0.61	0.19-2.39	0.13-5.48
Invert sugar[7]	mmol/100g FW	0.83	0.24-2.94	0.78	0.24-2.61	0.67	0.17-2.94	0.3-2.7
Amino-N[8]	mmol/100g FW	1.29	0.79-1.71	1.29	0.86-1.93	1.21	0.63-2.50	0.80-5.62

Legend:
[1] n = five sites, single analyses
[2] n = five sites, single analyses, eight commercial varieties
[3] References Märländer et al., 1996 and Smed et al., 1996.
[4] Polarization was determined using a polarimeter.
[5] Potassium was determined using a spectrophotometer.
[6] Sodium was determined using a spectrophotometer.
[7] Invert sugar was determined using the Institute of Berlin method.
[8] Amino-N was determined using a spectrophotometer.

C.3. Saponin analyses

Saponins are triterpenoid glycosides that occur naturally in numerous food and feed crops including beans, peas, potatoes, tea and sugar beet (Oakenfull and Sidhu, 1989). Hydrolysis of the glycoside releases a lipid-soluble sapogenin. The predominant sapogenin in sugar beet is oleanolic acid, whose structure is well characterized. Generally, saponins have a bitter and astringent taste and act as a deterrent to foraging. Saponins are actively eliminated during sugar processing and thus do not pose a risk to human health. Analysis for saponins in sugar beet usually consists of liberation of the oleanolic acid, which is quantified by HPLC (Ridout et al., 1994).

Root and top samples from event H7-1, the control and commercial reference varieties were analyzed for saponins using an HPLC method (Ridout et al., 1994). A data summary is presented in Table VI-16. Literature values from published sources that are presented in Table VI-16 establish the range currently accepted by the industry.

Table VI-16. Summary of saponin analyses of top and root tissues from event H7-1

		Control[1]		Event H7-1[1]		Reference Varieties[2]		Literature
Analysis[4]	Unit	Mean	Range	Mean	Range	Mean	Range	Range[3]
Top	mg/kg FW	65	47-100	58	34-93	76	27-200	50-600
Root	mg/kg FW	90	54-120	92	54-150	99	63-170	75-965

Legend:
[1] n = five sites, single analyses
[2] n = five sites, single analyses, eight commercial varieties
[3] Reference Lüdecke et al., 1958.
[4] Saponin was determined using a HPLC method.

The results indicate that saponin levels for event H7-1 are no different in comparison to the levels observed for the control and the levels expressed by the conventional reference varieties. Saponin mean levels for event H7-1 also fell within the published literature range for conventional varieties. It is concluded that event H7-1 does not differ from other commercially available sugar beet varieties with respect to saponin levels.

C.4. Amino acids

The levels of amino acids for sugar beet top and root samples are presented in Tables VI-17 and 18, respectively. The mean levels for fourteen of eighteen amino acids measured in top samples of event H7-1 were not significantly different than the mean levels of those same amino acids in the control top samples (Table VI-17). The mean levels observed for four amino acids, alanine, histidine, phenylalanine and tyrosine, in event H7-1 were statistically different when compared to the corresponding mean levels from the control; however, the ranges observed for these four amino acids from event H7-1 significantly overlapped or fell completely within the range of values observed for the control and the conventional reference varieties. Therefore, these differences are not considered to be biologically meaningful.

Table VI-17. Summary of amino acid analyses of top (leaf) tissue from event H7-1

Analysis[1]	Unit	Control[2]		Event H7-1[2]		Reference Varieties[3]	
		Mean	Range	Mean	Range	Mean	Range
Glutamic acid	% total aa	13.46	12.20-14.78	13.10	12.35-13.56	13.92	12.29-15.55
Alanine	% total aa	6.44	6.29-6.61	6.67*	6.29-7.07	6.53	5.98-6.97
Arginine	% total aa	5.36	5.13-5.69	5.44	5.06-5.91	5.42	4.68-5.96
Aspartic acid	% total aa	10.29	10.16-10.36	10.53	9.96-11.37	10.55	9.55-11.40
Cystine	% total aa	1.73	1.18-2.24	1.90	0.87-3.20	1.77	1.07-3.05
Glycine	% total aa	6.86	6.23-7.32	6.80	6.45-7.47	6.78	6.21-7.80
Histidine	% total aa	2.46	2.00-2.73	2.29*	1.73-2.54	2.26	1.75-2.68
Isoleucine	% total aa	4.49	4.26-4.86	4.39	4.12-4.67	4.44	4.11-4.97
Leucine	% total aa	8.20	7.58-9.19	8.13	7.66-9.06	8.01	7.16-9.01
Lysine	% total aa	5.36	4.82-5.81	5.25	3.75-5.81	5.35	4.60-5.85
Methionine	% total aa	1.83	1.37-2.45	2.20	1.13-4.05	1.74	1.06-3.18
Phenylalanine	% total aa	5.12	4.76-5.46	4.98*	4.65-5.30	4.99	4.47-5.40
Serine	% total aa	5.80	5.48-6.24	5.94	5.47-6.33	5.78	5.34-6.21
Threonine	% total aa	4.82	4.42-5.05	4.71	4.09-5.07	4.47	3.39-5.23
Thryptophane	% total aa	1.60	1.30-1.86	1.69	1.17-2.13	1.81	1.37-2.45
Tyrosine	% total aa	3.65	3.28-3.87	3.46*	3.05-3.69	3.63	3.21-4.70
Valine	% total aa	5.51	5.29-6.17	5.49	5.26-5.99	5.37	4.97-6.17
Proline	% total aa	7.04	5.53-7.95	7.04	6.55-7.47	7.15	5.46-9.58

Legend:
[1] Amino acids were determined using a HPLC method.
[2] n = five sites, single analyses
[3] n = five sites, single analyses, eight commercial varieties
* Indicates a statistically significant difference at the 5% level when compared with the corresponding control.

The mean levels for 16 of 18 amino acids measured in root samples of event H7-1 were not significantly different than the mean levels of those same amino acids in the control root samples (Table VI-18). However, the mean levels of two amino acids measured in event H7-1 root samples, alanine and glutamic acid, were statistically different than the mean levels of the same amino acids in the control root samples. The ranges for these two amino acids in event H7-1 significantly overlapped or fell completely within the range of values observed for the control and the conventional reference varieties. Therefore, these differences are not considered to be biologically meaningful.

Table VI-18. Summary of amino acid analyses of root tissue from event H7-1

		Control[2]		Event H7-1[2]		Reference Varieties[3]	
Analysis[1]	**Unit**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Glutamic acid	% total aa	18.58	15.66-21.85	16.87*	14.72-19.98	19.21	13.76-25.07
Alanine	% total aa	5.29	4.69-6.33	5.91*	5.04-6.73	6.27	4.73-9.43
Arginine	% total aa	4.91	4.50-5.18	5.30	4.94-5.63	4.80	4.02-5.74
Aspartic acid	% total aa	13.35	12.32-14.75	13.21	12.66-14.46	13.19	11.57-15.53
Cystine	% total aa	1.38	1.28-1.53	1.42	1.25-1.72	1.45	0.84-2.28
Glycine	% total aa	4.23	3.74-4.54	4.73	4.15-5.21	4.61	3.83-6.18
Histidine	% total aa	2.69	1.58-3.33	2.95	2.00-3.45	2.87	1.78-3.43
Isoleucine	% total aa	4.01	3.90-4.24	4.23	4.15-4.35	3.95	3.27-4.95
Leucine	% total aa	6.09	5.55-6.61	6.47	5.90-7.18	6.07	4.82-6.95
Lysine	% total aa	5.42	3.50-6.88	5.73	4.29-6.52	5.49	3.69-7.11
Methionine	% total aa	1.35	1.23-1.46	1.29	1.05-1.57	1.28	0.70-2.04
Phenylalanine	% total aa	3.36	2.98-3.69	3.45	3.29-3.66	3.26	2.75-3.75
Serine	% total aa	7.34	6.61-8.49	7.55	6.86-8.45	7.58	6.63-8.72
Threonine	% total aa	4.76	4.11-5.30	4.98	4.51-5.28	4.75	3.96-5.51
Thryptophane	% total aa	2.30	1.11-4.26	1.82	1.06-2.44	1.93	1.02-5.40
Tyrosine	% total aa	3.53	3.27-3.86	3.55	3.09-4.23	3.34	2.51-4.23
Valine	% total aa	5.47	5.10-5.82	5.14	3.79-5.87	4.84	1.99-7.49
Proline	% total aa	5.94	5.53-6.46	5.39	3.29-7.02	5.11	1.71-9.29

Legend:
[1] Amino acids were determined using a HPLC method.
[2] n = five sites, single analyses
[3] n = five sites, single analyses, eight commercial varieties
* Indicates a statistically significant difference at the 5% level when compared with the corresponding control.

C.5. Compositional and quality component conclusions

A comprehensive series of compositional and quality component analyses were conducted comparing the following components in event H7-1 to its control and a number of conventional sugar beet reference varieties. The analyses included:

- Five components of proximates, plus carbohydrate, of both top and root tissues,
- five nutrient quality components of brei (root tissue),
- saponin, in both top and root tissues, and
- 18 different amino acids in both top and root tissues.

A total of 55 statistical comparisons were made between event H7-1 and the near isogenic segregant control line for the top (leaf) and root (brei) tissues, with seven of these comparisons found to be statistically different at $p<0.05$. Of the seven comparisons found to be statistically different, 5%, or approximately three (0.05 X 55), were expected to be false positives based on chance alone. In section A.2, under the discussion of the comparator used in agronomic trials, which is the type of trial that produced the root and top samples, it was noted that the control used in this assessment was a "nearly-equivalent" hybrid that has similar germplasm characteristics. The inherent differences between the germplasms may have contributed to the minor compositional differences noted. Additionally, the values observed were likely influenced by a number of environmental factors including root maturity, harvest dates, plant and soil fertility, and weather (Smed et al., 1996). In all seven cases, however, the ranges for the statistically different components in event H7-1 significantly overlapped or fell completely within the range of values observed for the control, the conventional reference varieties and for available published values from conventional sugar beet varieties.

These results demonstrate that the levels of key nutrients and other nutritionally important components of event H7-1 are compositionally equivalent to top (leaf) and root (brei) tissues of the control and other conventional sugar beet varieties. The minor differences noted are not likely to be biologically meaningful and the top and root tissues of Roundup Ready sugar beet event H7-1 are considered to be no different than those of conventional sugar beets.

VII. ENVIRONMENTAL CONSEQUENCES AND AGRONOMIC PRACTICES

A. Current Agronomic Practices in Sugar Beet

Worldwide, approximately 30% of refined sugar is produced from sugar beet (FAO, 1999). In the U.S., sugar beet (*Beta vulgaris*) is an important crop, with approximately 1.3 and 1.4 million acres planted in the 2001 and 2002 seasons, respectively (USDA-NASS, 2002), which comprises approximately 10% of the world production. In 2000-2001, sugar beet production in the U.S. was valued at $1.025 to $1.113 billion (USDA-NASS, 2003a).

Sugar beet is a temperate climate biennial root crop typically grown in relatively dry, cool regions of the U.S. Planting occurs in the spring of the year for root production, whereas seed production involves planting in the fall of the year to allow the plants to go through a vernalization period prior to producing seed (see Section B in Part II). Harvesting of the sugar beet root occurs in the fall of the year for processing into sugar for food uses and molasses and pulp for feed uses. The harvested root is typically shipped directly to the processor, based on a contract agreement between the grower and processor. Whole beet roots can be processed at harvest or stored under cold conditions to await processing. Approximately 1,700 to 1,900 metric tons of sugar processed from sugar beet were exported in 2000-2001, respectively, with the exports valued at approximately $0.8 to $0.6 million, respectively. At the same time, approximately 600,000 to 675,000 metric tons of pulp processed from sugar beet were exported in 2000-2001, respectively, with the pulp exports valued at approximately $76.7 to $86.8 million (USDA-FAS, 2002).

Although agronomic practices for sugar beet production differ slightly across the U.S., there are several similar trends for most practices across the growing regions, including tillage to prepare the seed bed, and weed, insect, disease control and fertility management. The following sections describe the typical agronomic practices associated with sugar beet production in the major U.S. growing areas and the advantages of the Roundup Ready sugar beet system. Herbicide and rotational crop use information provided in this section is from communications with local agronomists and the USDA National Agricultural Statistics Service for the largest sugar beet producing states.

A.1. Sugar beet production in the U.S.

In 2002, approximately 1.4 million acres of sugar beet were planted in the 12 largest sugar beet production states in the U.S. (Table VII-1). Production areas are located in proximity to sugar production factories where the processing of beet roots into crystaline sugar occurs. Average root yield across the production areas in 2002 ranged from 18 to 39 tons per acre (Table VII-2). Sugar beet roots, at harvest, contain on average 16% sucrose, 80% of which can be recovered by the extraction process. Based on the 2002 average yield, it is expected that an acre of sugar beet production will produce 2.3 to 5.0 tons of processed refined sugar.

The primary objective of sugar beet growers in the U.S. is to economically produce root tonnage with high sugar content. Many variables can affect this production. Weather conditions during the production cycle can have a direct impact on sugar beet root yield. Sugar beet production typically requires about 22 inches of precipitation per acre during the growing season. Supplemental irrigation is used in areas where natural rainfall is limiting.

The primary concern for growers in sugar beet production is weed control. Sugar beet is a low growing plant which requires several weeks to reach canopy closure; weeds that are present prior to canopy closure or grow taller than the crop can compete agressively for the available moisture and nutrients. Dexter and Zollinger (2003) demonstrated in North Dakota and Minnesota field trials that a 5% loss per acre in extractable sucrose was realized with the presence of 25 redroot pigweed *(Amaranthus retroflexus)* or 30 wild oat (*Avena fatua*) plants per 100 feet of row length. In addition to the direct impact on yield, poorly managed sugar beet production fields with sufficient levels of weed pressure also impact harvest efficiency, reduce quality and increase the weed seed bank (Mesbah et al., 1994).

Due to the severe impact weeds can have on sugar beet production, weed control is considered by many growers to be their most serious problem (Dexter and Luecke; 2003). Herbicides are used extensively in sugar beet production (USDA-NASS, 2001). In the 2000 growing season, 13 different active ingredients formulated as various herbicide products were commonly used in U.S. sugar beet production, with a total of about 1.38 million pounds of herbicides applied (Table VII-3). Dexter and Luecke (2003) reported that 428% of the total sugar beet acreage was treated with herbicide applications in the survey area, indicating each acre of production receives multiple herbicide applications throughout the growing season.

Key weeds identified as problematic for growers include pigweed (*Amaranthus* spp.), lambsquarters (*Chenopodium album*) and kochia (*Kochia scorparia*). Current herbicide options often fail to provide adequate control of these key species. In the survey performed by Dexter and Luecke (2003), 29% of growers responded that they experienced fair or poor weed control from available postemergence herbicides.

Diseases of sugar beet are also an important consideration in production. Diseases which can limit production include those caused by viruses, bacteria, fungi and nematodes. Control of these diseases rely on resistant germplasm, crop rotations, cultural practices and chemical treatments (Wilson, 2001).

Table VII-1. Sugar beet: Area planted and harvested by state and United States, 2000-2002[1]

State	Area Planted (1,000 of acres)			Area Harvested (1,000 of acres)		
	2000	2001	2002	2000	2001	2002
California	98.0	46.8	50.0	93.5	44.7	48.0
Colorado	71.5	41.5	43.7	53.6	36.8	42.0
Idaho	212.0	199.0	210.0	191.0	179.0	209.0
Michigan	189.0	180.0	180.0	166.0	166.0	175.0
Minnesota	490.0	468.0	467.0	430.0	426.0	453.0
Montana	60.7	57.4	57.8	55.2	53.5	57.5
Nebraska	78.2	48.6	56.4	54.8	41.4	49.2
North Dakota	258.0	261.0	287.0	232.0	237.0	282.0
Ohio	1.2	0.8	1.7	0.8	0.6	1.7
Oregon	17.2	12.2	11.2	14.0	10.0	10.3
Washington	28.4	7.0	4.0	27.3	7.0	4.0
Wyoming	61.0	48.5	40.0	56.1	41.6	39.0
US Totals	**1,565.2**	**1,370.8**	**1,408.8**	**1,374.3**	**1,243.6**	**1,370.7**

[1] Source: National Agricultural Statistics Service (www.usda.gov/nass)

Table VII-2. Sugar beet: Total root production and yield by state and United States, 2000-2002[1]

State	Total Root Production (1,000 tons)			Yield (tons per acre)		
	2000	2001	2002	2000	2001	2002
California	3,039	1,618	1,862	32.5	36.2	38.8
Colorado	1,206	824	800	22.5	22.4	19.7
Idaho	5,596	4,636	5,313	29.3	25.6	25.3
Michigan	3,403	3,220	3,150	20.5	19.4	18.0
Minnesota	9,245	7,796	8,563	21.5	18.3	19.2
Montana	1,319	1,150	1,140	23.9	21.5	20.0
Nebraska	1,112	840	767	20.3	20.3	18.0
North Dakota	5,127	4,290	5,254	22.1	18.1	18.5
Ohio	17	12	34	21.0	20.0	20.0
Oregon	413	291	320	29.5	29.1	29.4
Washington	803	253	150	29.4	36.1	37.5
Wyoming	1,156	857	675	20.6	20.6	18.5
US Totals	**32,436**	**25,787**	**28,028**	**23.6**	**20.7**	**20.7**

[1] Source: National Agricultural Statistics Service (www.usda.gov/nass)

Table VII-3. Sugar Beet: Agricultural chemical applications, United States, 2000[1]

Agricultural Chemical (Herbicide)	Area Applied (%)	Number of Applications	Rate per Application (lb./acre)	Rate per Crop Year (lb./acre)	Total Applied (1,000 lbs.)
Clethodim	46	2.5	0.04	0.11	77
Clopyralid	74	2.8	0.03	0.09	102
Cycloate	5	1.0	1.84	1.84	139
Desmedipham	94	2.8	0.07	0.18	270
EPTC	6	1.0	2.61	2.64	230
Ethofumesate	37	2.1	0.06	0.14	82
Glyphosate	13	1.1	0.39	0.43	86
Phenmedipham	80	2.6	0.05	0.14	170
Pyrazon	6	1.0	0.82	0.85	76
Quizalofop-p-ethyl	10	1.6	0.04	0.06	9
Sethoxydim	11	1.7	0.19	0.33	56
Trifluralin	5	1.0	0.65	0.66	55
Triflusulfuron	83	2.7	0.008	0.02	29

[1] 1.565 million acres were planted in the U.S. in 2000.
Source: National Agricultural Statistics Service (www.usda.gov/nass)

A.2. Conclusions

Sugar beet is a crop that requires extensive management during production, yet it provides major economic benefits to growers and the sugar industry. Managing weeds in sugar beet production is considered by growers to be the most difficult aspect of sugar beet production. As the dynamics of sugar beet production change, it is evident that new technologies, including varieties improved by modern biotechnology, will play an important role in the evolution of agricultural production practices. Though some agronomic practices such as cropping systems, crop rotations, seed bed preparation and cultivation options will continue to be important considerations in sugar beet production.

B. Agronomic Impact of Roundup Ready Sugar Beet

A number of broadleaf and grass weeds regularly infest sugar beet production fields and weed control is needed to achieve optimum yields. Weed infestations are considered by a majority of growers to be the most serious problem they face in cultivation of sugar beet (Dexter and Luecke, 2003). Herbicides are a key input component of sugar beet production in the U.S., with approximately 98% of planted acres receiving one or more herbicide applications in 2000 (USDA-NASS, 2001). Current weed control strategies in sugar beet cultivation are complex. Herbicides, or multiple applications of mixtures of

herbicides, plus the use of applicable cultural control techniques (hand weeding) are required to effectively control all weeds (Dexter and Luecke, 2003). Current agronomic practices for weed control in sugar beet include tillage, pre-plant incorporation of grass and broadleaf herbicides, and in-crop use of grass and broadleaf herbicide tank mixtures (Dexter and Luecke, 2003; Dexter and Zollinger, 2003; WSSA, 1994). Each of these practices has limitations. Cultivation and pre-plant incorporation of herbicides can increase soil erosion and in-crop applications of post-emergent herbicides are associated with limited windows of application (Baker et al., 1982; Baker and Johnson, 1979; Campbell and Janzen, 1995; CTIC, 2000; Fawcett, 1995; Zollinger, 2000). Additionally, herbicide performance and crop injury are influenced heavily by soil pH, target weed size, crop size, air temperature and irrigation practices. Many of the currently applied herbicides leave soil residues, whose persistence can impact crop rotation options in subsequent seasons (Dexter and Zollinger, 2003; WSSA, 1994).

B.1. Benefits of the Roundup Ready sugar beet system

Use of the Roundup Ready sugar beet system - that is, planting Roundup Ready sugar beet event H7-1 and applying a Roundup agricultural herbicide - offers a number of potential advantages over the complex practices used in current weed control and will offer growers new opportunities for effective and economical production of sugar beet. The advantages and benefits that the Roundup Ready sugar beet system will bring to U.S. sugar beet production are described in the following sections.

B.1.a. Effective and broad spectrum weed control. Roundup agricultural herbicides have been used extensively for nonselective weed control in various cropping systems for over two decades. A more comprehensive discussion of glyphosate-based herbicides is provided by Baird (1971), Malik et al. (1989) and Franz et al. (1997). No other product has been shown to provide the weed spectrum, overall efficacy and consistency seen with Roundup agricultural herbicides. To illustrate this, Table VII-4 compares Roundup agricultural herbicide with the typical sugar beet herbicides currently used and their expected efficiacy against common weeds found in sugar beet production fields in North Dakota. This consistency and efficacy have been demonstrated in Roundup Ready sugar beet field trials with both the currently deregulated Roundup Ready sugar beet event 77 and event H7-1, where weed control was similar to or better than current weed control practices utilizing multiple herbicides (Kniss et al., 2003; Wilson et al., 2002). In other field research, the Roundup Ready sugar beet system using two applications of a Roundup agricultural herbicide provided equivalent control of ragweed and foxtail, with higher sugar beet yields, compared to three applications of desmedipham, triflursulfuron and clopyralid, plus one application of sethoxydim (Gianessi et al., 2002). Likewise, Gianessi et al. (2002) reported that the Roundup Ready sugar beet system using two applications of a Roundup agricultural herbicide provided 100% control of ALS-resistant kochia, whereas three applications of ethofumesate, phenmedipham, triflusulfuron, clopyralid and clethodim gave only 82% control. The two applications of glyphosate also provided 100% control of wild oats and black nightshade.

Table VII-4. Relative herbicide effectiveness on weeds. Modified from 2003 North Dakota Weed Control Guide[1], Circular W-253. Solid black boxes indicate good to excellent control; open boxes, fair to poor or no control for the weeds indicated.

Herbicide[2,3]	Red Root Pigweed	Green Foxtail	Yellow Foxtail	Lambs-quarters	Kochia	Wild Oat	Wild Buckwheat	Wild Proso Millet	Common Mallow	Common Cocklebur	Wild Mustard	Venice Mallow	Eastern Black Nightshade
Roundup	■	■	■	■	■	■		■	■	■	■	■	■
Assure II		■	■			■		■					
Betamix	■			■	■						■		■
Betanex	■			■							■		■
Eptam/Eradicane (PPI)[4]	■	■	■			■		■	■				
Nortron SC (PPI)	■	■	■		■	■	■						■
Nortron SC (PRE)[5]	■					■							
Poast		■	■			■		■					
Progress	■	■	■	■	■		■				■		■
Pyramin	■			■	■						■		■
Ro-Neet	■	■	■	■		■							■
Select		■	■			■		■					
Stinger							■			■		■	■
Treflan (PPI)	■	■	■	■								■	
Treflan (PoPI)	■	■	■	■									
UpBeet + Betanex/Betamix/Progress	■	■	■	■	■		■		■	■	■	■	■

[1] Table excerpted from information in the 2003 North Dakota Weed Control Guide, found at http://www.ag.ndsu.nodak.edu/weeds/w253/w253w.htm and the 2003 Crop Protection Reference (www.greenbook.net).

[2] Active ingredients are as follows: Assure II: quizalofop-p-ethyl; Betamix: phenmedipham + desmedipham; Betanex: desmedipham; Eptam/Eradicane: EPTC; Nortron SC: ethofumesate; Poast: sethoxydim; Progress: desmedipham + phenmedipham + ethofumesate; Pyramin: pyrazon; Ro-Neet: cycloate; Select: clethodim; Stinger: clopyralid; Treflan: trifluralin; and UpBeet: triflusulfuron.

[3] Weed control is dependent on rate used, size of weed, environmental conditions, and number of applications.

[4] PPI = Preplant Incorporated application

[5] PRE = Preemergent application

[6] PoPI = Postplant Incorporated application

CBI-Deleted

B.1.b. Crop safety. Herbicides currently used in sugar beet production can cause crop injury (Table VII-5), particularly when applied at the incorrect rate or crop stage or under stressful environmental conditions (Dexter and Luecke, 2000a; Dexter 2000; Dexter and Luecke, 2000b; WSSA, 1994). The Roundup Ready sugar beet system has demonstrated outstanding crop safety, especially as it relates to yield in field trials (Kniss et al., 2003; Wilson et al., 2002). Wilson et al. (2002) determined, with the currently deregulated Roundup Ready sugar beet event 77, that in comparison to a standard treatment regime involving three sequential applications with several conventional herbicides, two sequential applications of a Roundup agricultural herbicide utilized in the Roundup Ready sugar beet system resulted in sucrose yield that was about 15% higher than the standard commercial herbicide program. Likewise, Kniss et al. (2003) determined that event H7-1 treated with three applications of a Roundup agricultural herbicide produced greater root yield than five of six different conventional and micro-rate herbicide treatments, and produced greater gross sucrose yield than all six conventional treatments. In research reported by Gianessi et al. (2002), field trials conducted in North Dakota and Minnesota using the Roundup Ready sugar beet system with two applications of a Roundup agricultural herbicide produced less crop injury than sugar beets treated with three applications of desmedipham, triflursulfuron and clopyralid, plus one application of sethoxydim. Likewise, Gianessi et al. (2002) reported that the Roundup Ready sugar beet system employing sequential treatments of a Roundup agricultural herbicide resulted in less early season crop injury than a conventional weed control program in Nebraska. With the implementation of the Roundup Ready sugar beet system, growers will experience a reduction in the potential for herbicide crop injury, along with greater weed control, and will be have the ability to maximize yield.

B.1.c. Simplicity and flexibility of use. Many herbicide products currently in use have a narrow window of application, which is based on a specific weed size or crop stage. Additionally, current weed control options involve a high degree of complexity due to varying application timings required by the multiple herbicides applied to achieve broad spectrum weed control. As an example, a common practice in sugar beet production is to use a micro-rate herbicide strategy for weed control (Dexter and Zollinger, 2003). This is accomplished by tank mixing multiple herbicides at reduced rates in combination with a methylated seed oil adjuvant. The components of the tank mixture typically include combinations of herbicides comprised with the active ingredients desmedipham, phenmedipham and ethofumosate with triflusulfuron and clopyralid, as well as a graminicide, such as clethodim, if grasses are present. A minimum of three applications is recommended, beginning at the cotyledon growth stage and followed by weekly applications of this herbicide mixture. The intent of the micro-rate program is to lower overall herbicide costs and reduce the potential for crop injury.

Table VII-5. Herbicide information[1] relative to chemical weed control options in sugar beet production

Trade Name (Manufacturer)	Active Ingredient (Mode of Action)	Application Window	Remarks	Comments
Roundup Ultra (Monsanto)	glyphosate (EPSPS inhibitor)	Post[2]	Excellent broad spectrum weed control.	Ammonium salt can improve performance with hard water.
Eptam (Syngenta)	EPTC[3] (lipid synthesis inhibitor	Pre/PPI[2]	Some stand reductions. Temporary stunting.	More injury and damage seen on sandy soils with low organic matter.
Ro-Neet (Syngenta)	cycloate (lipid synthesis inhibitor)	Pre/PPI	Incorporation improves performance.	Activate with irrigation if no rainfall within 5-10 days after application. Less effective on high organic soils (greater than 5%).
Nortron (Bayer CropSciences)	ethofumesate	Pre/PPI	Temporary stunting. Soil incorporation improves field performance.	Crop rotation restrictions the following season against production of sunflower, soybeans, wheat, barley and oats.
Pyramin (Micro Flo)	pyrazon	Pre/PPI	Soil incorporation improves field performance.	Less effective on soils with organic matter > 5%.
Betamix (Bayer CropSciences)	desmediphan & phennediphan	Post	Sugar beet injury can be severe.	Apply when daytime highs are not expected to go above 80 °F.

CBI-Deleted

Table VII-5 (continued). Herbicide information[1] relative to chemical weed control options in sugar beet production

Trade Name (Manufacturer)	Active Ingredient (Mode of Action)	Application Window	Remarks	Comments
Stinger (Dow AgroSciences)	clopyralid (growth regulator)	Post	Pre-harvest interval of 105 days.	Crop rotation restrictions against growing alfalfa, canola, sweet and popcorn, soya, beans and sunflowers the following season.
Select (Valent)	clethodim (Acc-ase inhibitor)	Post	Always apply with an oil additive.	Grass control only.
Assure II (DuPont)	quizalofop (Acc-ase inhibitor)	Post	Annual grass control only.	Apply with petroleum oil adjuvant.

Legend:

[1] Herbicide information taken from Dexter (2000) and WSSA (1994) with manufacture's names updated from the 2003 Crop Protection Reference (www.greenbook.net).

[2] Pre = preplant application, PPI = preplant incorporated, Post = post emergent application to weed species

[3] EPTC = ethyl diprorylthiocarbamate

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In-crop applications of a Roundup agricultural herbicide can be made from crop emergence up to 30 days prior to harvest. This flexibility will allow the grower a wider window of application, with the application timing based on weed pressure, not on crop stage. The broad spectrum of weed control offered by Roundup agricultural herbicides (Table VII-4) will reduce the need for tank mixing with additional herbicides, and provide growers with an excellent tool for weed control in Roundup Ready sugar beet.

B.1.d. Environmental benefits of Roundup agricultural herbicides compared to alternative herbicides. There are established benefits associated with the use of Roundup agricultural herbicides, which will also be realized in the Roundup Ready sugar beet system, compared to alternative herbicides currently used by sugar beet producers. Glyphosate has documented favorable characteristics with regard to risk to human health, non-target species, and the environment (Malik et al., 1989; Geisy et al., 2000; Williams et al., 2000). Glyphosate is classified by the EPA as Catergory E (evidence of non-carcinogenicity for humans) (57 FR 8739). In 1998, the EPA granted Reduced Risk status for an expedited review of the submitted residue data package supporting the use of glyphosate, as Roundup Ultra herbicide (EPA Registration No. 524-475), for use in Roundup Ready sugar beets. Reduced Risk status was granted by EPA based on a detailed hazard comparison of glyphosate to alternative herbicides available for weed control in sugar beet production (Reduced Risk petition document: MRID 44560501), and an overall conclusion that weed control with Roundup Ultra herbicide offers a substantial benefit to sugar beet growers in the form of reduced risk to human health, non-target species and the environment.

Monsanto and KWS believe that this conclusion is also applicable to the application of Roundup agricultural herbicides to Roundup Ready sugar beet event H7-1. Presented below is a similar, but abbreviated, comparative analysis of the hazard/risk characteristics of glyphosate, the active ingredient in Roundup UltraMAX® herbicide (EPA Registration No. 524-512), to the most commonly used herbicides applied in sugar beet production, based on total pounds of active ingredient applied (USDA-NASS, 2001). A detailed assessment of the potential chronic human health risks compared to alternative products will not be presented in this comparison. The assessment is based on information obtained from various sources, including product-specific labeling, EPA Reregistration Eligibility Documents (RED), EPA RED Fact Sheets, product-specific Federal Register publications, the EPA Ecotoxicology One-Liner database, the USDA Pesticide Properties database, and other public sources of product-specific toxicological and environmental profile information.

The following assessment shows that in the majority of cases, weed control with glyphosate, as Roundup UltraMAX herbicide, in the Roundup Ready sugar beet system offers the benefit of less risk for applicators and handlers of concentrated product from potential exposure and a reduced potential to impact non-target species and water quality.

® Roundup UltraMAX is a registered trademark of Monsanto Technology LLC.

Comparative toxicity: Table VII-6 provides a comparison of product-specific labeling, including required precautionary statements associated with acute exposure hazards, as well as statements associated with environmental risk concerns. Although most alternative products carry the same Signal Word (Caution!) as the Roundup UltraMAX herbicide label, the associated precautionary statements for all of the alternative herbicide products are indicative of toxicity findings that represent a greater acute exposure risk. More importantly, every alternative product evaluated here has more restrictive requirements for the use of Personal Protective Equipment (PPE) than those required for Roundup UltraMAX herbicide in order to mitigate the risks of acute exposure, and in some cases to mitigate risks associated with longer-term or chronic exposure for applicators and handlers.

Impact on non-target species: For non-target terrestrial species, available ecological assessments in EPA REDs provide the support that the use of glyphosate represents a reduction in chronic risk to birds compared to trifluralin and a reduction in acute risk to small mammals in comparison to EPTC. The product label for Select 2EC (clethodim) warns of risk to federally listed endangered plant species. For all the other alternative herbicide products, as well as glyphosate, no significant risks to birds or other non-target terrestrial species were indicated in the available information.

For non-target aquatic species, Tables VII-7, VII-8 and VII-9 provide summaries of the estimated exposure and hazard information for the alternative herbicides currently used in sugar beet production, and present quantitative comparisons of the derived Risk Quotients. Exposure, defined as the expected environmental concentration (EEC), was estimated for all products using the standard assumptions of 5% drift of spray applied to a one-acre field onto water and 5% runoff from 10 treated acres into a one-acre pond six feet in depth. Herbicide treatments were based on the maximum single application rate taken from product labels. Hazard information (LC_{50} or EC_{50}) for each active ingredient was taken from the EPA Ecotoxicology One-Liner Database (if available) and summarized in Tables VII-7, VII-8 and VII-9 as the upper and lower values from the range of values reported. Hazard information for the end-use formulated products is generally not readily available, thus this analysis is a comparison based solely on the active ingredients. Any label warnings and other available hazard and/or risk descriptions for non-target aquatic species are also included. The Risk Quotient is determined for each active ingredient by divding the EEC by the hazard (LC_{50} or EC_{50}) value.

The labels for products containing desmedipham, phenmedipham, sethoxydim, clethodim and trifluralin include warnings of toxicity or adverse effects to fish, and/or aquatic invertebrates and/or aquatic plants. Risk Quotients that exceed the Trigger Value[1] of 0.5 are highlighted in bold text in Tables VII-7, VII-8 and VII-9 as a Level of Concern, based on EPA Ecological Effects Rejection Analysis. Alternative herbicide products containing triflusulfuron, desmedipham, trifluralin and pyrazon are shown to exceed this Level of

[1] The trigger Value of 0.5 for acute aquatic Risk Quotients was established in EPA's Ecological Effects Pesticide Rejection Rate Analysis document, dated December 1994, page 4.

Concern.

Impact on water quality: Table VII-10 presents a comparison of parameters that are indicative of water contamination potential for glyphosate and the commonly used alternative herbicides in sugar beet prodcution. Summary information on soil dissipation rate and soil binding for alternative herbicides was taken from the USDA Pesticide Properties Database or, if not available there, from WSSA (1994). The Groundwater Ubiquity Scores (GUS) were calculated as a comparable indicator of leaching potential (Gustafson, 1989). For glyphosate, the soil dissipation and soil binding data were taken from the original Monsanto reports cited in the glyphosate RED (MRIDs 108192 and 42607501)[2]. The GUS indices, as shown in Table VII-10, are used to group herbicide active ingredients into High (H), Medium (M) or Low (L) categories for potential leaching. Although the potential for herbicide runoff into surface water is not directly related to a GUS value, the general association between high use rates, longevity in soil, low soil binding, and a potential for runoff is valid.

This analysis concludes that glyphosate has a Low (L) potential for leaching or runoff. Of the alternative herbicides in this comparison with available information, seven herbicides: clopyralid, cycloate, desmedipham, EPTC, ethofumesate, pyrazon and quizalofop, are shown to have a Medium (M) or High (H) potential risk for leaching based on their GUS values. Although information was not available to calculate the GUS index for clethodim, its product labeling does carry a warning statement for water contamination through runoff. It can be concluded that the use of Roundup agricultural herbicides for weed control in the Roundup Ready sugar beet system would offer a potential reduction in ground water and surface water contamination compared to the currently used herbicide products.

Cumulative comparison of Roundup agricultual herbicides: The comparative analyses provided in this section are summarized in a table (Table VII-11) that indicates those areas for which glyphosate (designated with a ✓), using Roundup UltraMAX herbicide in the comparison, offers the benefit of potential risk reduction compared to the most commonly used alternative herbicides in sugar beet production. In this cumulative comparison, glyphosate offers potential benefits over all the alternative sugar beet herbicides in at least two and up to six risk assessment categories. These comparisons demonstrate the benefits to applicators, mixers and non-target organisms from the use of Roundup agricultural herbicides in the Roundup Ready sugar beet system.

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[2] For glyphosate, since soil binding is likely an ionic phenomenon not correlated with organic matter content, the use of Koc values to characterize soil binding is somewhat artifactual. However, for this comparison, the high Koc values properly reflect the extremely tight binding of glyphosate to most soils, although it is not likely caused by their organic matter component.

Table VII-6. Alternative herbicides for weed control in sugar beets - label comparison / exposure mitigation

Active Ingredient(s)	Product Brand	Label Signal Word	Sugar Beet PHI (days)	Max. lb ai/acre (single appl.)	Max. lb. ai/acre (season)	Label Precautionary Statements / Special Directions / Other Information	Applicator and Handler PPE Required to Mitigate Exposure Risks
Glyphosate	Roundup UltraMAX	Caution	30	0.75	8	Causes moderate eye irritation. Do not store in steel. Four resistant weed biotypes confirmed to date.	Long sleeved shirt, long pants, shoes plus socks.
Clethodim[a]	Select 2 EC or Prisim	Warning	40	0.09	0.25	Causes substantial, but temporary, eye injury. Harmful if swallowed or inhaled. Potential skin sensitizer. Warnings and precautions for runoff and drift. Use of the product may pose hazard to ferderally listed endangered plant species. Warnings for repeated use leading to selection of resistant weed biotypes. Crop injury warnings.	Long sleeved shirt, long pants, shoes plus socks, chemical resistant gloves, protective eyewear. Do not reuse heavily contaminated clothing.
Clopyralid	Stinger	Caution	45	0.25	0.25	Causes eye injury. Harmful if inhaled or absorbed through skin. Warning for leaching to groundwater under certain conditions. Crop injury warnings for 1) use of treated plant material or manure from animals grazed in treated areas, as mulch or compost; and 2) spreading of treated soil. Up to 18-month rotation restrictions to many crops due to risk of injury; field bioassay recommended.	Long sleeved shirt, long pants, water proof gloves, shoes plus socks.

Table VII-6 (continued). Alternative herbicides for weed control in sugar beets - label comparison / exposure mitigation

Active Ingredient(s)	Product Brand	Label Signal Word	Sugar Beet PHI (days)	Max. lb ai/acre (single appl.)	Max. lb. ai/acre (season)	Label Precautionary Statements / Special Directions / Other Information	Applicator and Handler PPE Required to Mitigate Exposure Risks
Cycloate	Ro-neet	Caution	NA; preplant incorporation	4	4	Harmful if swallowed. Avoid contamination of food or feed. Soil incorporation or soil injection required. Crop injury concerns dependent on soil type.	Long sleeved shirt, long pants, chemical resistant gloves, shoes plus socks. Added PPE in California for a non-closed system; for mixers/loaders: chemical resistant clothing, full face respirator; for applicators: coveralls, plus half-face respirator, 93 gallon limit for 21-day period.
Desmedipham[b]	Betanex	Caution	75	1.2	1.92	Harmful if swallowed. Causes moderate eye irritation. Prolonged or frequent repeated skin contact may cause allergic reaction. This product contains the toxic inert ingredient isophorone. This product is toxic to fish. Do not apply where runoff is likely to occur. Sugar beet injury possible under many situations.	Long sleeved shirt, long pants, chemical resistant gloves, shoes plus socks, protective eyewear.

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Table VII-6 (continued). Alternative herbicides for weed control in sugar beets - label comparison / exposure mitigation

Active Ingredient(s)	Product Brand	Label Signal Word	Sugar Beet PHI (days)	Max. lb ai/acre (single appl.)	Max. lb. ai/acre (season)	Label Precautionary Statements / Special Directions / Other Information	Applicator and Handler PPE Required to Mitigate Exposure Risks
Desmedipham/ phenmedipham	Betamix	Warning	75	1.2	1.92	Causes substantial, but temporary, eye injury. Harmful if swallowed or absorbed through skin. This product contains the toxic inert ingredient isophorone. This product is toxic to fish and aquatic organisms. Drift and runoff...may be hazardous to fish and aquatic organisms... Physical hazard: Combustible. Sugar beet injury possible under many situations; evening applications recommended. Rotation restriction of 120 days for cereals.	Long sleeved shirt, long pants, chemical resistant gloves, shoes plus socks, protective eyewear.
EPTC[c]	Eptam	Caution	NA; preplant incorporation or very early postemergence	4.2	5.6	Harmful if swallowed. Avoid breathing spray mist. Incorporation or soil injection required unless applied through irrigation.	Long sleeved shirt, long pants, chemical resistant gloves, shoes plus socks.
Ethofumesate	Nortron	Caution	90	3.6	4	Harmful if swallowed, inhaled or absorbed through skin. Rotation restrictions of 6 to 12 months for crops other than sugar beets or ryegrass. Do not graze livestock on treated crops.	Long sleeved shirt, long pants, water proof gloves, shoes plus socks.

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Table VII-6 (continued). Alternative herbicides for weed control in sugar beets - label comparison / exposure mitigation

Active Ingredient(s)	Product Brand	Label Signal Word	Sugar Beet PHI (days)	Max. lb ai/acre (single appl.)	Max. lb. ai/acre (season)	Label Precautionary Statements / Special Directions / Other Information	Applicator and Handler PPE Required to Mitigate Exposure Risks
Pyrazon	Pyramix DF	Caution	0	7.3	7.3	Harmful if swallowed, inhaled or absorbed through skin. Avoid breathing dust or spray mist. Causes moderate eye irritation. Significant crop injury warning statements, depending on soil moisture level, soil type (organic matter content, loam, sandy, etc.), temperature at time of application, application method, and tank mix products.	Long sleeved shirt, long pants, chemical resistant gloves, shoes plus socks. Do not reuse clothing heavily contaminated with this product's concentrate.
Quizalofop-p-ethyl	Assure II	Danger	45 days, except 60 days for feeding of tops	0.17	0.17	Causes severe eye irritation. May irritate skin, nose and throat. May be harmful if absorbed through skin, swallowed or inhaled. This product contains petroleum-based distallates. Rotation restriction of 120 days for crops not labeled. Need spray adjuvant added.	Long sleeved shirt, long pants, chemical resistant gloves, shoes plus sock, protective eyewear. Do not reuse clothing heavily contaminated with this product's concentrate.
Sethoxydim[d]	Poast	Warning	60	0.40	0.8	Causes substantial, but temporary, eye injury. Harmful if swallowed. This product is toxic to aquatic organisms. Crop injury warnings. Multiple confirmed resistant weed biotypes.	Coveralls over short sleeved shirt and short pants, chemical resistant gloves, chemical resistant footwear, protective eyewear, chemical resistant headgear for overhead exposure, chemical resistant apron for clearning, mixing, loading.

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Table VII-6 (continued). Alternative herbicides for weed control in sugar beets - label comparison / exposure mitigation

Active Ingredient(s)	Product Brand	Label Signal Word	Sugar Beet PHI (days)	Max. lb ai/acre (single appl.)	Max. lb. ai/acre (season)	Label Precautionary Statements / Special Directions / Other Information	Applicator and Handler PPE Required to Mitigate Exposure Risks
Trifluralin[e]	Treflan HFP	Caution	NA; one application between first true leaf and 6 inch stage	0.72	4.0	Causes moderate eye irritation, harmful if swallowed, potential skin sensitizer. This product contains aromatic hydrocarbon and can be extremely toxic if swallowed. This pesticide is extremely toxic to freshwater marine and estuarine fish and aquatic invertebrates. Soil incorporation required within 24 hrs of application. Crop injury warnings. Crop rotation restrictions ranging from 5 to 21 months. Confirmed multiple resistant weed biotypes.	Long sleeved shirt, long pants, shoes plus socks, chemical resistant gloves, protective eyewear. Do not reuse clothing heavily contaminated with this product's concentrate.
Triflusulfuron	Upbeet	Caution	60	0.008	0.08	Resistant weed biotypes; multiple MOA resistance. Need spray adjuvant added.	Long sleeved shirt, long pants, chemical resistant gloves, shoes plus socks.

NA indicates not applicable.

[a] Based on recent clethodim tolerance action (67 FR 46893, Final Rule, July 17, 2002) percent-crop-treated market data necessary to refine chronic dietary exposure estimates; per label statement: concern for risks to endangered plant species.

[b] 1996 desmedipham RED: concern for Margins of Exposure (MOE) for dermal exposure to mixers and loaders; additional concern for applicator inhalation exposure to wettable-powder formulations requiring limits on application rate per acre and number of acres treated per day; low to moderate chronic risk to birds.

[c] 1999 EPTC RED ; 10x FQPA UF retained due to neurotoxic effects; developmental neurotoxicity study required; reversible Cholinesterase inhibitor; Tier 3 refinements using average residues and percent crop treated data for chronic dietary assessment; concern for risk to applicators and handlers from dermal and inhalation exposure; concern for risks to small mammals and non-target plants, including endangered species from run-off and spray drift.

[d] Based on recent sethoxydim tolerance actions (66 FR 51587, Final Rule, Oct.10, 2001), aPAD includes additional 3x FQPA UF for acute exposure to females 13+ yrs of age, due to fetal effects seen in rat developmental tox study; anticipated residues and percent-crop-treated data necessary to refine chronic exposure assessment.

[e] 1995 Trifluralin RED: concern for cancer risk to applicators, handlers and field workers; moderately to highly toxic to fish and aquatic invertebrates; chronic risk concern for birds due to evidence of egg cracking in avian study.

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Table VII-7. Comparison of potential effects of glyphosate and alternative sugar beet herbicides on fish

Active Ingredient	Max. lb/acre (single appl.)	EEC[1] (ppm)	Fish LC_{50} (a.i.)[2] Range (ppm)		Fish Risk Quotient[3] Range		Classification / Label Warnings
			low	high	worst	best	
Glyphosate	0.75	0.025	85	130	0.0003	0.0002	
Clethodim	0.09	0.003	19	>33	0.0002	<0.0001	
Clopyralid	0.25	0.008	104	125	0.0001	0.0001	
Cycloate	4.0	0.134	4.5	7	0.03	0.02	
Desmedipham	1.2	0.040	1.7	6	0.024	0.007	Toxic to fish.
EPTC	4.2	0.141	11.5	27	0.012	0.005	
Ethofumesate	3.6	0.121	2.5	>320	0.048	<0.0004	
Phenmedipham	0.6	0.020	1.41	3.98	0.014	0.005	
Pyrazon	7.3	0.245	NA	NA	NA	NA	
Quizalofop-p-ethyl	0.17	0.006	0.46	10.72	0.013	0.001	
Sethoxydim	0.47	0.016	170	265	0.0001	0.0001	Toxic to aquatic organisms.
Trifluralin	0.72	0.024	0.008	0.2	**3.0**	0.12	Extremely toxic to freshwater, marine and estuarine fish.
Triflusulfuron	0.08	0.003	730	760	0.000004	0.000004	

Legend:
NA = information not available
[1] EEC refers to the Expected Environmental Concentration, which assumes 5% drift from a one-acre field and 5% runoff from a 10-acre field to a one-acre pond six feet deep.
[2] Aquatic LC_{50} values obtained from the EPA Ecotoxicology One-Liner Database.
[3] Risk Quotient is EEC/LC_{50}. Risk Quotient **Bolded** if > 0.5 = Level of Concern [criteria from EPA Ecological Effects, Rejection Analysis]

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Table VII-8. Comparison of potential effects of glyphosate and alternative sugar beet herbicides on aquatic invertebrates

Active Ingredient	Max. lb/acre (single appl.)	EEC[1] (ppm)	Invertebrate EC_{50} (a.i.)[2] Range (ppm)		Invertebrate Risk Quotient[3] Range		Classification / Label Warnings
			low	high	worst	best	
Glyphosate	0.75	0.025	780	NA	0.00003	NA	
Clethodim[4]	0.09	0.003	20.2	NA	0.0001	NA	
Clopyralid	0.25	0.008	132	NA	0.0001	NA	
Cycloate	4.0	0.134	2.6	24	0.052	0.006	
Desmedipham	1.2	0.040	1.88	NA	0.021	NA	
EPTC	4.2	0.141	0.63	66	0.224	0.002	
Ethofumesate	3.6	0.121	64	294	0.02	0.0004	
Phenmedipham	0.6	0.020	3.2	NA	0.006	NA	Toxic to fish and aquatic organisms.
Pyrazon	7.3	0.245	NA	NA	NA	NA	
Quizalofop-p-ethyl	0.17	0.006	0.15	6.4	0.04	0.001	
Sethoxydim	0.47	0.016	78	NA	0.0002	NA	Toxic to aquatic organisms.
Trifluralin	0.72	0.024	0.037	2.2	**0.65**	0.01	Extremely toxic to aquatic invertebrates.
Triflusulfuron	0.08	0.003	960	NA	0.000003	NA	

Legend:
NA = information not available or not applicable
[1] EEC refers to the Expected Environmental Concentration, which assumes 5% drift from a one-acre field and 5% runoff from a 10-acre field to a one-acre pond six feet deep.
[2] Aquatic Invertebrate EC_{50} values obtained from the EPA Ecotoxicology One-Liner Database.
[3] Risk Quotient is EEC/EC_{50}. Risk Quotient **Bolded** if > 0.5 = Level of Concern [criteria from EPA Ecological Effects, Rejection Analysis]
[4] EC_{50} value is from a study using a 25.6% ai concentration.

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Table VII-9. Comparison of potential effects of glyphosate and alternative sugar beet herbicides on aquatic plants (algae and duckweed)

Active Ingredient	Max. lb/acre (single appl.)	EEC[1] (ppm)	Aquatic Plant EC_{50} (a.i.)[2] Range (ppm)		Aquatic Plant Risk Quotient[3] Range		Classification / Label Warnings
			low	high	worst	best	
Glyphosate	0.75	0.025	0.9	39.9	0.028	0.001	
Clethodim	0.09	0.003	1.34	>11.4	0.0022	<0.0003	May pose hazard to federally listed endangered plants.
Clopyralid	0.25	0.008	NA	NA	NA	NA	
Cycloate	4.0	0.134	NA	NA	NA	NA	
Desmedipham	1.2	0.040	0.044	>0.33	**0.909**	<0.121	
EPTC	4.2	0.141	1.36	41	0.101	0.003	
Ethofumesate	3.6	0.121	2.76	NA	0.043	NA	
Phenmedipham	0.6	0.020	NA	NA	NA	NA	Toxic to aquatic organisms.
Pyrazon	7.3	0.245	0.17	>4.6	**1.441**	<0.053	
Quizalofop-p-ethyl	0.17	0.006	0.083	>1.77	0.073	<0.003	
Sethoxydim	0.47	0.016	NA	NA	NA	NA	Toxic to aquatic organisms.
Trifluralin	0.72	0.024	0.015	5.0	**1.60**	0.005	Extremely toxic to aquatic invertebrates.
Triflusulfuron	0.08	0.003	0.003	0.123	**1.00**	0.02	

Legend:
NA = information not available or not applicable
[1] EEC refers to the Expected Environmental Concentration, which assumes 5% drift from a one-acre field and 5% runoff from a 10-acre field to a one-acre pond six feet deep.
[2] Aquatic EC_{50} values obtained from the EPA Ecotoxicology One-Liner Database.
[3] Risk Quotient is EEC/EC_{50}. Risk Quotient **Bolded** if > 0.5 = Level of Concern [criteria from EPA Ecological Effects, Rejection Analysis]

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Table VII-10. Comparison of potential effects of glyphosate and alternative sugar beet herbicides on water quality

Active Ingredient	Field Soil Dissipation DT_{50} (days)		Soil Binding[3] K_{OC}		Ground Water Ubiquity Assessment[1]		Comments
	Range	Nominal Value[2]	Range	Nominal Value[2]	GUS Value	Classification	
Glyphosate	2 - 142	15	1,823 – 11,667	5500	0.3	L	
Clethodim	NA	3	NA	NA	NA	NA	Label precautions for preventing water contamination from run off and drift.
Clopyralid	10 – 30	13	6 - 36	36	**2.7**	**M**	Label warnings for leaching and groundwater contamination.
Cycloate	12 - 56	27	41 - 430	272	**2.2**	**M**	
Desmedipham	NA	30	95 - 210	150	**2.7**	**M**	
EPTC	6 – 32	18	170 - 280	223	**2.1**	**M**	Special concern for off-site movement and water contamination due to volatility and drift; detections in rain samples collected in Minnesota; some potential to leach; groundwater detections; and new field soil dissipation study required by 1999 RED.
Ethofumesate	20 - 150	80	120 - 760	276	**3.0**	**H**	
Phenmedipham	16 - 55	25	1,166 – 14,000	7,500	0.2	L	
Pyrazon	NA	21	NA	120	**2.5**	**M**	
Quizalofop-p-ethyl	NA	60	510 - 570	540	**2.3**	**M**	
Sethoxydim	1 - 10	5	NA	100	1.4	L	
Trifluralin	15 - 132	81	1,200 – 13,700	7,200	0.3	L	Not a leacher; binds tightly.
Triflusulfuron	2.9 – 6.6	3.3	32 - 150	60	1.2	L	

Legend:

NA = information not available or not applicable

[1] In the ground water assessment, the GUS (Groundwater Ubiquity Score) value can be used to assess leaching potential. A GUS value < 1.8 represents a low (L) leaching potential; a GUS value >1.8, but < 2.8 represents a medium (M) leaching potential; and a GUS value > 2.8 represents a high (H) leaching potential. A GUS value is **Bolded** if it is determined to be medium or high, indicating increased potential of impacting water quality compared to glyphosate.

[2] Nominal value in most cases refer to the "suggested value" in the USDA ARS Pesticide Properties Database at http://www.arsusda.gov/ppdb.htm. If not available in the USDA database, values are from the WSSA Herbicide Handbook, Seventh Ed., 1994. For glyphosate, "nominal value" refers to the mean value taken from MRIDs 108192 or 42607501 cited in the Glyphosate RED.

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Table VII-11. Potential reduction in risk from use of Roundup agricultural herbicides compared to alternative herbicides used in U.S. sugar beet production

Active Ingredients[1]	Human Health Risk		Non-Target Species Risks					Groundwater Contamination	Total Number of Areas for Potential Risk Reduction
	Acute	Chronic	Mammals	Fish	Aquatic Invertebrates	Aquatic Plants	Avian		
Clethodim	✓	✓				✓		✓	4
Clopyralid	✓							✓	2
Cycloate	✓							✓	2
Desmedipham	✓	✓		✓		✓	✓	✓	6
EPTC	✓	✓	✓					✓	4
Ethofumesate	✓							✓	2
Phenmedipham	✓			✓	✓	✓			4
Pyrazon	✓					✓		✓	3
Quizalofop-p-ethyl	✓							✓	2
Sethoxydim	✓	✓		✓	✓	✓			5
Trifluralin	✓	✓		✓	✓	✓	✓		6
Triflusulfuron	✓					✓			2

Legend:
[1] Alternative herbicides are compared to glyphosate, using the label from Roundup UltraMAX herbicide.
✓ Indicates there is a potential for reduction in risk category by using Roundup agricultural herbicides.

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B.1.e. Projected economic impact. Weed control in sugar beet production with herbicides is expensive, costing growers in 2001 an average of $78.09 per acre, which ranged from a low of $47.15 per acre in Nebraska to a high of $105.93 per acre in Montana (Doane, 2003). These expenses do not reflect the additional costs associated with hand weeding or cultivation, both of which are common in sugar beet production. When including the cost of hand weeding and cultivation, the average cost of weed control increases to $136 per acre (Gianessi et al., 2002). The Roundup Ready sugar beet system is expected to reduce the cost of weed control in sugar beet production an estimated $60 per acre when compared to the currently available weed control options (Gianessi et al., 2002). In a separate assessment on the economic impact of the Roundup Ready sugar beet system compared to conventional herbicides, substantial cost differences were determined (Kniss et al., 2003). In the assessment by Kniss et al., three applications of a Roundup agricultural herbicide to event H7-1 were compared to six different conventional treatments applied to a "nearly equivalent" variety as the control. Based on the cost of the Roundup agricultural herbicide treatment and the recoverable sugar, it was determined that a producer using event H7-1 would have a $479 per hectare ($194 per acre) advantage, averaged over the herbicide treatments and minus any cost for the Roundup Ready sugar beet seed. In additon to reducing the cost of weed control, Roundup agricultural herbicides will provide broad spectrum weed control, reducing the need for hand weeding and cultivation. The impact of cultivation itself has been shown to reduce overall yield of sugar beet production (Dexter et al., 2000), and therefore the reduced need for cultivation will provide growers the potential benefit of increased yield with the use of the Roundup Ready sugar beet system.

B.1.f. Resistant weed management. The current weed control strategies in sugar beet production are complex, requiring the use of multiple herbicides with various modes of action to control the spectrum of weeds associated with sugar beet production, including herbicide-resistant weed species. A herbicide-resistant weed is one that develops resistance to a herbicide, commonly due to the selective pressure of repeated applications of a herbicide, typically at sublethal rates, or due to a herbicide's residual nature and is then able to pass the aquired trait to its offspring. ALS-resistant *Kochia scoparia* and *Amaranthus retroflexus* are two weeds of concern to sugar beet growers, where effective weed control with current commercial products is limiting. Table VII-12 lists resistant weeds identified in major sugar beet producing states. Management of herbicide-resistant weeds is an important consideration for sugar beet growers. The Roundup Ready sugar beet system will provide a new mode of action, with a broad spectrum of activity, that will reduce the selection pressure on the present herbicide-resistant weeds and offer an alternative means of controlling established resistant populations of these weeds. For a discussion on glyphosate-resistant weeds, refer to Appendix 1.

Table VII-12. Herbicide-resistant weeds identified in key sugar beet producing states in the U.S.[1]

Idaho

Species	Common Name	Year[2]	Herbicide Class
1. *Lactuca serriola*	Prickly Lettuce	1987	ALS inhibitors
2. *Kochia scoparia*	Kochia	1989	ALS inhibitors
3. *Salsola iberica*	Russian Thistle	1990	ALS inhibitors
4. *Avena fatua*	Wild Oat	1992	ACCase inhibitors
5. *Lolium multiflorum*	Italian Ryegrass	1992	ACCase inhibitors
6. *Avena fatua*	Wild Oat	1993	Thiocarbamates
7. *Avena fatua*	Wild Oat	1993	Pyrazoliums
8. *Anthemis cotula*	Mayweed Chamomile	1997	ALS inhibitors
9. *Kochia scoparia*	Kochia	1997	Synthetic Auxins

Michigan

Species	Common Name	Year[2]	Herbicide Class
1. *Chenopodium* album	Lambsquarters	1975	Photosystem II inhibitors
2. *Ambrosia artemisiifolia*	Common Ragweed	1990	Photosystem II inhibitors
3. *Senecio vulgaris*	Common Groundsel	1990	Photosystem II inhibitors
4. *Portulaca oleracea*	Common Purslane	1991	Photosystem II inhibitors
5. *Portulaca olercea*	Common Purslane	1991	Ureas and amides
6. *Daucus carota*	Wild Carrot	1993	Synthetic Auxins
7. *Ambrosia artemisiifolia*	Common Ragweed	1998	ALS inhibitors
8. *Amaranthus tuberculata*	Tall Waterhemp	2000	ALS inhibitors
9. *Amaranthus powellii*	Powell Amaranth	2001	Photosystem II inhibitors
10. *Amaranthus powellii*	Powell Amaranth	2001	Ureas and amides
11. *Amaranthus retroflexus*	Redroot Pigweed	2001	Photosystem II inhibitors
12. *Amaranthus retroflexus*	Redroot Pigweed	2001	Ureas and amides
13. *Chenopodium album*	Lambsquarters	2001	ALS inhibitors
14. *Polygonum persicaria*	Ladysthumb	2001	Photosystem II inhibitors
15. *Amaranthus hybridus*	Smooth Pigweed	2002	ALS inhibitors
16. *Conyza canadensis*	Horseweed	2002	ALS inhibitors
17. *Conyza canadensis*	Horseweed	2002	Photosystem II inhibitors
18. *Conyza canadensis*	Horseweed	2002	Ureas and amides

Minnesota

Species	Common Name	Year[2]	Herbicide Class
1. *Chenopodium album*	Lambsquarters	1982	Triazines
2. *Abutilon theophrasti*	Velvetleaf	1991	Triazines
3. *Amaranthus retroflexus*	Redroot Pigweed	1991	Triazines
4. *Avena fatua*	Wild Oat	1991	ACCase inhibitors
5. *Kochia scoparia*	Kochia	1994	ALS inhibitors
6. *Xanthium strumarium*	Common cocklebur	1994	ALS inhibitors
7. *Setaria faberi*	Giant Foxtail	1996	ALS inhibitors
8. *Setaria viridis*	Robust White Foxtail (var. robusta-alba Schreiber)	1996	ALS inhibitors
9. *Setaria lutescens*	Yellow Foxtail	1997	ALS inhibitors
10. *Ambrosia artemisiifolia*	Common Ragweed	1998	ALS inhibitors

Table VII-12 (continued). Herbicide-resistant weeds identified in key sugar beet producing states in the U.S.[1]

North Dakota

Species	Common Name	Year[2]	Herbicide Class
1. *Kochia scoparia*	Kochia	1987	ALS inhibitors
2. *Setaria viridis*	Green Foxtail	1989	Dinitroanilines and others
3. *Avena fatua*	Wild Oat	1991	ACCase inhibitors
4. *Kochia scoparia*	Kochia	1995	Synthetic Auxins
5. *Avena fatua*	Wild Oat	1996	ALS inhibitors
6. *Kochia scoparia*	Kochia	1998	Triazines
7. *Amaranthus retroflexus*	Redroot Pigweed	1999	ALS inhibitors
8. *Sinapis arvensis*	Wild Mustard	1999	ALS inhibitors
9. *Solanum ptycanthum*	Eastern Blk.Nightshade	1999	ALS inhibitors

Legend:

[1] Source: Heap, I. The International Survey of Herbicide-resistant Weeds. Online. Internet. Available at www.weedscience.com.

[2] Year resistance was first reported.

B.2. Impact of Roundup Ready sugar beet on current rotational practices

Crop rotation following sugar beet production is a key component of a sound sugar beet production strategy. Currently, the primary reasons for crop rotation are to aid in the management of diseases and pests that affect sugar beet and to serve as a weed management tool. The introduction of Roundup Ready sugar beet will not change crop rotation systems, nor is it expected to significantly alter the rotational crop patterns associated with current sugar beet production, given the continued need to manage diseases and pests common in sugar beet production.

To determine the typical rotational crops planted after sugar beet production, the rotational practices followed in the primary production states were assessed. The top nine states identified in Table VII-13 represent about 99% of the total U.S. sugar beet production acreage, with the typical rotational crops planted after sugar beet production provided on a state-by-state basis. These rotational crops include alfalfa, barley, corn, dry beans, durum wheat, oats, potato, soybean, spring wheat and winter wheat. Barley, corn, soybean and spring wheat account for about 91% of the rotational crops based on acreage. A sugar beet-to-sugar beet rotation is uncommon due to the increased likelihood of disease (such as *Cercospora* leaf spot and *Aphanomyces* root rot) and pests (such as nematodes) in the subsequent sugar beet crop. The one exception occurs in Wyoming, where approximately 10% of the sugar beet production acres are rotated back to sugar beets (Table VII-13).

Currently, other commerically available Roundup Ready crops are grown in sugar beet production states and Monsanto is petitioning USDA-APHIS for a determination of nonregulated status for additional Roundup Ready crops. Therefore, some of the rotational crops following Roundup Ready sugar beet production may be another

Roundup Ready crop. Corn and soybeans are two crops that currently have significant adoption rates with Roundup Ready varieties, where approximately 9% of the U.S. corn and 75% of the U.S. soybean acres planted in 2002 were Roundup Ready (USDA-NASS, 2003b). Roundup Ready alfalfa and spring wheat, and sugar beet on limited acreage in Wyoming, will be rotational crop options available to sugar beet growers in the future. The adoption rate of these new Roundup Ready crops, for this discussion, is assumed to be 50%. To gain a better understanding of the extent to which Roundup Ready sugar beet acreage could be rotated to another Roundup Ready crop, an assessment was performed based on crop rotational data from the primary sugar beet production states. This assessment was performed on the basis of the total rotational crop acreage throughout the major sugar beet production states, since this will establish the extent within each of these states to which successive Roundup Ready crops can be planted due to the commercialization of Roundup Ready sugar beet.

First, the acreage of sugar beet production in each state is expressed as a percentage of the total rotational crop acreage to indicate whether sugar beet is the primary crop preceding each rotational crop. Based on the rotational crop acreage (Table VII-13), the percentage of sugar beet preceding each state's total rotational crops (column J) was calculated to range from 0.6% in Nebraska to 16.5% in Wyoming, demonstrating that sugar beet acreage, even in the primary sugar beet production states, is only a small portion of the total acreage. The percentage of the total rotational crops rotated from Roundup Ready sugar beet to another Roundup Ready crop (column K) in these states is also anticipated to be low, ranging from 0.1% in Montana to 3.76% in Idaho. Overall, sugar beet production represents only 2.89% of the total rotational crop acreage, with only 1.19% of the total states' rotational crop acreage anticipated to be Roundup Ready sugar beet rotated to another Roundup Ready crop. Given these assessments on the primary sugar beet production states, on an annual basis approximately 1% of the total rotational crop acres are expected to be a Roundup Ready crop rotated from the production of event H7-1.

A recent report shows that neither the percentage of growers adopting the Roundup Ready sugar beet system nor the extent of rotational Roundup Ready crop acreage following Roundup Ready sugar beet production will result in an increased likelihood of developing glyphosate-resistant weeds. Research summarized in the Nebraska Farmer (Wilson and Stahlman, 2003) reported that crop rotations of Roundup Ready corn, Roundup Ready sugar beet and Roundup Ready spring wheat treated at the recommended Roundup UltraMAX herbicide use rate over a five-year period resulted in fewer weed escapes compared to the same rotations treated with either non-glyphosate herbicides or alternating non-glyphosate herbicides with Roundup UltraMAX herbicide every other year. The research also concluded there was no evidence of glyphosate-resistant weeds developing after five years of continuous use of Roundup UltraMAX herbicide on these Roundup Ready crops.

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Table VII-13. Rotational crops following U.S. sugar beet production and an estimation of rotational crops as Roundup Ready crops
These rotational crop data are based on Monsanto's estimates from 2002 plantings. All acreages are expressed as 1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	Total Sugar Beet Acres[1]	Major Crops That Follow Sugar Beet In Rotation[2]	Total Acreage of Rotation Crop in States[1]	Percent of Rotational Crop Rotated From Sugar Beet[3]	Rotational Crop Acres Following Sugar Beet[2]	Percent Rotational Crop of Total Sugar Beet[4]	Percent Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	Percent of Sugar Beet Acres Preceeding Major Rotations[7]	Estimated Percentage of Roundup Ready Crops as Major Rotations[8]
CA	50	Barley	130	7.7%	10	20%	NA	0		
		Dry Beans	92	5.4%	5	10%	NA	0		
		Durum	95	10.5%	10	20%	NA	0		
		Oats	260	3.8%	10	20%	NA	0		
		Spring Wheat	530	2.8%	15	30%	50%	7.5		
			Total: 1,107		**Total: 50**			**Total: 7.5**	**4.52%**	**0.68%**
CO	44	Barley	85	5%	4	10%	NA	0		
		Corn	1200	3%	31	70%	9%	2.8		
		Dry Beans	92	8%	7	15%	NA	0		
		Potato	78	3%	2	5%	NA	0		
			Total: 1,455		**Total: 44**			**Total: 2.8**	**3.02%**	**0.19%**
ID	212	Alfalfa	730	1.5%	11	5%	50%	5.5		
		Barley	730	4.4%	32	15%	NA	0		
		Corn	190	3.2%	6	3%	9%	0.5		
		Dry Beans	95	4.2%	4	2%	NA	0		
		Spring Wheat	530	30.0%	159	75%	50%	79.5		
			Total: 2,275		**Total: 212**			**Total: 85.5**	**9.32%**	**3.76%**
MI	180	Corn	2,250	5.2%	117	65%	9%	10.5		
		Dry Beans	270	6.7%	18	10%	NA	0		
		Soybean	2,050	2.2%	45	25%	75%	33.8		
			Total: 4,570		**Total: 180**			**Total: 44.3**	**3.94%**	**0.97%**
MN	505	Barley	210	23.8%	50	10%	NA	0		
		Soybean	7,200	4.9	354	70%	75%	265.5		
		Spring Wheat	2,000	5.1%	101	20%	50%	50.5		
			Total: 9,410		**Total: 505**			**Total: 316**	**5.37%**	**3.36%**

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Table VII-13 (continued). Rotational crops following U.S. sugar beet production and an estimation of rotational crops as Roundup Ready crops

These rotational crop data are based on Monsanto's estimates from 2002 plantings. All acreages are expressed as 1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	Total Sugar Beet Acres[1]	Major Crops That Follow Sugar Beet In Rotation[2]	Total Acreage of Rotation Crop in States[1]	Percent of Rotational Crop Rotated From Sugar Beet[3]	Rotational Crop Acres Following Sugar Beet[2]	Percent Rotational Crop of Total Sugar Beet[4]	Percent Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	Percent of Sugar Beet Acres Preceeding Major Rotations[7]	Estimated Percentage of Roundup Ready Crops as Major Rotations[8]
MT	**58**	Barley	1,200	2.4%	29	50%	NA	0		
		Corn	65	21.5%	14	25%	9%	1.3		
		Dry Beans	27	33.3%	9	15%	NA	0		
		Spring Wheat	3,750	0.2%	6	10%	50%	3		
			Total: 5,042		**Total: 58**			**Total: 4.3**	**1.15%**	**0.09%**
ND	**265**	Barley	1,600	2.5%	40	15%	NA	0		
		Corn	1,230	3.3%	40	15%	9%	3.6		
		Durum	2,100	0.6%	13	5%	NA	0		
		Soybean	2,670	4.0%	106	40%	75%	79.5		
		Spring Wheat	6,900	1.0%	66	25%	50%	33		
			Total: 14,500		**Total: 265**			**Total: 116.1**	**1.83%**	**0.80%**
NE	**57**	Corn	8,400	0.3%	29	50%	9%	2.6		
		Dry Beans	185	11.9%	22	40%	NA	0		
		Winter Wheat	1,650	0.4%	6	10%	NA	0		
			Total: 10,235		**Total: 57**			**Total: 2.6**	**0.56%**	**0.03%**
WY	**40**	Barley	90	24.4%	22	55%	NA	0		
		Corn	80	12.5%	10	25%	9%	0.9		
		Dry Beans	32	12.5%	4	10%	NA	0		
		Sugar Beet	40	10%	4	10%	50%	2.0		
			Total: 242		**Total: 40**			**Total: 2.9**	**16.53%**	**1.20%**

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Table VII-13 (continued). Rotational crops following U.S. sugar beet production and an estimation of rotational crops as Roundup Ready crops
These rotational crop data are based on Monsanto's estimates from 2002 plantings. All acreages are expressed as 1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Sugar Beet Acres[1]**	**Major Crops That Follow Sugar Beet In Rotation[2]**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Sugar Beet[3]**	**Rotational Crop Acres Following Sugar Beet[2]**	**Percent Rotational Crop of Total Sugar Beet[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Sugar Beet Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Overall	**1,411**	Alfalfa	730	1.5%	11	0.8%	50%	5.5		
		Barley	4,045	4.6%	186	13.2%	NA	0		
		Corn	13,415	1.8%	247	17.5%	9%	22.2		
		Dry Beans	793	8.7%	69	4.9%	NA	0		
		Durum	2195	1.0%	23	1.6%	NA	0		
		Oats	260	3.8%	10	0.7%	NA	0		
		Potato	78	2.6%	2	0.1%	NA	0		
		Soybean	11,920	4.2%	505	35.8%	75%	378.8		
		Spring Wheat	13,710	2.5%	347	24.6%	50%	173.5		
		Sugar Beet	40	10%	4	0.3%	50%	2.0		
		Winter Wheat	1,650	0.4%	6	0.4%	NA	0		
State Totals			**Total: 48,836**		**Total: 1,411**			**Total: 582.0**	**2.89%**	**1.19%**

Legend:
NA denotes not applicable.
[1] Acreage planted of the specific crop is based on 2002 planting data (USDA-NASS, 2003b).
[2] Rotated crops and acreage following sugar beet production are based on communications from individual local experts, i.e., university agronomists, USDA-ARS and Monsanto field personnel.
[3] Column E obtained by dividing Column F by Column D and multiplying by 100.
[4] Column G obtained by dividing Column F by Column B and multiplying by 100.
[5] Roundup Ready rotational crop penetration rates for corn and soybean are based on 2002 plantings (USDA-NASS, 2003b); penetration rates for alfalfa, spring wheat and sugar beet are assumed to be 50% for the purpose of this assessment.
[6] Column I obtained by multiplying Column F by Column H.
[7] Column J obtained by dividing Column B by Column D Total and multiplying by 100.
[8] Column K obtained by dividing Column I Total by Column D Total and multiplying by 100.

B.3. Management of Roundup Ready sugar beet volunteers

Management of volunteer sugar beet plants is typically not necessary following sugar beet production, due mainly to the sugar beet plant's growth habit. Sugar beet is a biennial plant that is predomintely grown as an annual for vegetative root production, as discussed in Part II. Volunteers from root production rarely occur in the sugar beet production states. The occassional volunteer plant, known as a ground keeper or weed beet, grows up from residual root material in the soil after harvest. Ground keepers are cold sensitive and do not easily survive the winter conditions found in most sugar beet production states. In the unlikey situation that an event H7-1 ground keeper plant were to survive the winter, it would not present a new management problem for growers, as glyphosate is not the only herbicide used to control volunteers.

As described in section B.2, the major crops rotated with conventional sugar beet production today are barley, corn, soybean and spring wheat. Depending on the rotation crop chosen to follow sugar beet, growers can continue to utilize the current option(s) of tillage and/or herbicide treatments with the active ingredients methylsulfuron methyl, 2,4-dichlorophenoxyacetic acid (2,4 D) and 3,6-dichloro-*o*-anisic acid (dicamba) for the control of volunteer ground keepers prior to planting and after crop emergence. In 2002, herbicide-tolerant corn and soybean represented 9% and 75%, respectively, of the total planted acreage of these crops in the U.S. (USDA-NASS, 2003b). Since their introduction in 1996, the management of herbicide-tolerant volunteers of these crops has been successful using established control practices, such as alternative herbicides and tillage. An effective management strategy for the occassional volunteer Roundup Ready sugar beet ground keeper is expected to utilize the same or similar control practices.

In the U.S., biennial growth of the sugar beet plant occurs during seed production, which is currently limited to the state of Oregon. Additionally, sugar beet seed production is conducted under contract, where the grower follows recommendations to control volunteers resulting from seed production. The seed production recommendations state that once the sugar beet seed has been harvested, the field is to be managed with a regime of repeated shallow tillage followed by irrigation, or natural precipitation, in the fall to allow emergence of sugar beet plants. The emerged sugar beet plants are controlled with tillage or herbicide treatments, as many times as needed, until a killing frost. The field is fallowed over the winter until a spring planting of another crop, with any new sugar beet volunteer plants in the spring controlled with either tillage or herbicides containing the active ingredients methylsulfuron methyl, 2,4-dichlorophenoxyacetic acid (2,4 D) and 3,6-dichloro-*o*-anisic acid (dicamba). Occasionally, a harvested sugar beet seed field is planted immediately to fall seeded annual rye grass or wheat. In these fall rotations, the volunteer sugar beet plants are controlled with established alternative herbicides (Table 14).

Table VII-14. Herbicide control of sugar beet volunteers in fall rotations after seed production

Agronomic Rotation	Active Ingredient
Fallow	2,4 D
Annual Rye Grass	bromoxynil + MCPA
	dicamba
Wheat	thifensulfuron-methyl + tribenuron-methyl
	dicamba

In summary, volunteer control in sugar beet root production will involve controlling the occassional volunteer ground keeper plant with either: 1) frost, 2) herbicides containing methylsulfuron methyl, 2,4 D or dicamba, or 3) tillage. In sugar beet seed fields, limited to Oregon, volunteers will be controlled with established practices followed by contracted growers, involving either: 1) germination of remnant seed followed by control with light tillage, herbicide applications and/or a killing frost and 2) herbicide application in fallow or fall-seeded grass crops.

C. Cross Pollination to Sugar Beet Relatives

Pollination of *Beta vulgaris* is predominately a wind-mediated and, to a much lesser extent, insect-mediated process. Sugar beet is considered a strongly self-incompatible plant during pollination, due to the stigma not being fully mature when the flower opens (OECD, 2001), which results in the need for cross-pollination with other sugar beet or *Beta* sp. plants for seed production. As discussed in Part II, the pollen grains produced by the anther are round and typically have numerous indentations, with an anther producing about 17,000 grains of pollen. The survival of the dispersed pollen grains is limited to a maximum of 24 hours, depending on environmental conditions, such as temperature and humidity (OECD, 2001).

Pollen-mediated gene flow from one plant species to another (e.g., sugar beet to another species) can only occur if the following requirements are met: 1) the two species are sexually compatible; 2) both species flower at the same time; and 3) the species are growing in proximity of one another. In Europe, pollen-mediated gene flow between sugar beet (*B. vulgaris*) to wild and weedy *Beta* species has been determined (Desplanque et al., 1998). The potential for development of U.S. populations of sugar beet relatives that are resistant to Roundup agricultural herbicides as a result of pollen-mediated gene transfer from event H7-1 is not likely to occur due to geographic and agronomic barriers, as discussed in the following Sections C.1. and C.2.

C.1. Geographic factors influencing gene flow

Sugar beet is known to hybridize freely with other members of the *Beta* genus, including wild relatives (OECD, 2001). In the U.S., there are few wild relatives present that can cross pollinate with sugar beet. Of the wild relatives in the U.S. that can interbreed with sugar beets, populations of *B. vulgaris* ssp. *maratima* and *B. macrocarpa* are present in

California, with evidence that *B. macrocarpa* has hybridized with cultivated sugar beet (Bartsch and Ellstrand, 1999). These are the only two wild relatives of sugar beet present in the U.S., and these populations are limited to California (Appendix 2). One population exists in the San Francisco Bay area, a region where there is no sugar beet production. The second population, taxonomically classified as *Beta macrocarpa*, is found in the Imperial Valley and is considered a weed in sugar beet. In the opinions of Drs. Panella and Llewellyn (Appendix 2), it is highly unlikely that gene flow between cultivated herbicide-tolerant sugar beet and *B. macrocarpa*, or any other wild beet species, will result in hybrid plants in the U.S. that will become a weed problem. This is based on the assessment that *B. macrocarpa* has a much earlier flowering period compared to sugar beet, with no significant overlap, resulting in temporal isolation in production fields. Also, an assessment of greenhouse generated F_1 hybrid plants demonstrated they were mainly male sterile and subsequent F_2 hybrid plants had very disturbed genetic ratios and growth habits, which would not support survival of hybrid plants in nature.

This conclusion by Drs. Panella and Llewellyn is also supported by the fact that large scale sugar beet seed multiplication, with the dispersal of viable pollen from the biennial growth habit, takes place mainly in the Willamette Valley of Oregon, where there are no known wild beet species. On an annual basis, approximately 3,000 to 5,000 acres of seed production takes place in Oregon. Considering that wild relatives of cultivated sugar beet are located exclusively in California, gene flow between wild relatives and sugar beet cultivated for seed multiplication is not expected to occur.

C.2. Agronomic factors minimizing gene-flow potential

In addition to the geographic factors influencing gene flow, there are agronomic factors which impact the potential gene flow in sugar beet. In sugar beet seed multiplication conducted in Oregon, contracted growers are selected for their ability to follow Oregon Seed Certification Standards. These standards require a minimum isolation distance of 3,200 feet between sugar beet varieties and at least 8,000 feet from other *Beta* species, including fodder beet, red beet and swiss chard. These isolation distances would be expected to contain the movement of pollen (wind/insects) which is reported to occur at distances less than 1,000 meters (3,280 feet) (Dark, 1971).

Pollen-mediated gene flow from sugar beet root production is inherently low, since sugar beet plants are grown for their vegetative root. Environmental conditions can occur though during sugar beet root production to induce the plant to flower, commonly referred to as bolting (refer to Part II). Bolting in sugar beet root production usually occurs as a result of the early planting of bolting sensitive sugar beet varieties, with low vernalization requirements, followed by cold conditions during the early stages of plant development. Additionally, ground keepers, discussed in Section B, that over winter have the ability to bolt the next season if not controlled. The presence of either of these types of bolting plants, with their potential for pollen dispersal, would compete for the available nutrients and moisture, thereby negatively impacting root production. Therefore, agronomic practices are currently utilized to control bolters.

As discussed in Part VI Section B, seed producers conduct coded trials to meet industry guidelines and performance criteria prior to market release of a new variety. In the process of replacing bolting-sensitive varieties, coded trials are conducted to select new cultivars with a reduced bolting characteristic. This practice has resulted in more recently developed varieties that are generally less susceptible to bolting (see Appendix 2). In the event that bolters do occur in root production fields, due either to bolting susceptible varieties or ground keepers that over winter, fields are scouted for bolters and bolting plants are manually removed from the production field. This agronomic practice, in conjunction with new varieties selected against bolting, greatly reduces the potential of pollen shed in sugar beet root production.

If successful pollen-mediated gene flow were to occur between Roundup Ready sugar beet event H7-1 and other *Beta* species, the resultant hybrid plants could still be controlled with either tillage or herbicides containing the active ingredients methylsulfuron methyl, 2,4 D and dicamba, as described in Section B.

C.3. Conclusions on cross pollination to sugar beet relatives

Cross pollination between cultivated sugar beet to sexually compatible *Beta* species requires that both species flower at the same time and that they are located in close proximity to one another for fertilization to occur. Cross pollination of Roundup Ready sugar beet event H7-1 to other *Beta* species or wild relatives can be managed in the U.S. by the geographic barriers and agronomic practices that serve to effectively limit, in seed production, or greatly reduce, in root production, the occurrence of pollen-mediated gene flow. The following summarizes the discussion above:

Geographic barriers that reduce or effectively limit hybridization:
- Wild relatives of sugar beet, *Beta* sp., in the U.S. are limited to California.
- Certified sugar beet seed is currently produced only in Oregon.

Agronomic barriers that reduce or limit hybridization:
- Seed production is contracted with growers who follow seed certification standards, including isolation distances that are designed to contain wind- or insect-mediated pollen movement.
- Seed producers ensure new varieties meet industry guidelines and performance criteria, such as bolting potential, prior to market release.
- Sugar beet plants have a biennial growth habit with pollen production occurring after a vernalization period, and most U.S. acreage is managed for the vegetative root.
- Bolters present in root production fields are manually removed, limiting the potential for pollen to be shed in root production fields.

VIII. ADVERSE CONSEQUENCES OF INTRODUCTION

It is concluded that Roundup Ready sugar beet event H7-1 is no more likely to pose a plant pesk risk than conventional sugar beet, based on the following: 1) pest susceptibility observations confirmed that event H7-1 is no more susceptible to diseases or insect pests than conventional sugar beet; 2) agronomic characteristics, performance and different morphological data have demonstrated that event H7-1 is not meaningfully different morphologically and agronomically than conventional sugar beet, indicating there is no competitiveness or weediness difference between event H7-1 and conventional sugar beet; 3) compositional and quality component analyses have shown that event H7-1 is comparable to its conventional control for key compositional and quality constituents; and 4) the only phenotypic difference observed between event H7-1 and conventional sugar beet is the presence of the CP4 EPSPS protein, which confers glyphosate tolerance. As such, the *cp4 epsps* gene, including the regulatory sequences, and the CP4 EPSPS protein do not confer plant pest characteristics to event H7-1.

Considering the above, Monsanto and KWS know of no unfavorable grounds associated with event H7-1, and no adverse consequences of its introduction are expected. Therefore, on the basis of the substantial benefits to the grower and the environment, Monsanto and KWS request that Roundup Ready sugar beet event H7-1 and all progeny derived from this event no longer be regulated under 7 CFR part 340.

IX. REFERENCES

Asgrow. 2002. Asgrow Seed Company Product Guide: Disease ratings by Asgrow Seed Company breeders.

Baerson, S.R., D.J. Rodriguez, M. Tran, Y. Feng, N.A. Biest and G.M. Dill. 2002. "Glyphosate-resistant Goosegrass. Identification of a Mutation in the Target Enzyme 5-Enolpyruvylshikimate-3-Phosphate Synthase," Plant Physiology 129:1265-1275.

Baird, D.D., R.P. Upchurch, W.B. Homesley and J.E. Franz. 1971. Introduction of a new broad spectrum post emergence herbicide class with utility for herbaceous perennial weed control. North. Cent. Weed Control Conf. 26:64-68.

Baker, J.L., J.M. Laflen and R.O. Hartwig. 1982. Effects of corn residue and herbicide placement on herbicide runoff losses. Transactions of the American Society of Agricultural Engineers 0001-2351/82/2502-0340. pp. 340-343.

Baker, J.L. and H.P. Johnson. 1979. The effect of tillage systems on pesticides in runoff from small watersheds. Transactions of the American Society of Agricultural Engineers 0001-2351/79/2203-0554. pp. 554-559.

Barker, R.F., K.B. Idler, D.V. Thompson and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octapine Ti plasmid pTi15955. Plant Mol. Biol. 2:335-350.

Barry, G., G. Kishore, S. Padgette, M. Taylor, K. Kolacz, M. Weldon, D. Re, D. Eichholtz, K. Fincher and L. Hallas. 1992. Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants. pp. 139 - 145. *In* Biosynthesis and Molecular Regulation of Amino Acids in Plants, Singh et al., (eds.), American Society of Plant Physiologists.

Bartsch, D. and N.C. Ellstrand. 1999. Genetic evidence for the origin of California wild beet (genus *Beta*). Theor. Appl. Genet. 99:1120-1130.

Bolivar, F., R.L. Rodriguez, P.J. Green, M.C. Betlach, H.L. Heyneker and H.W. Boyer. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene 2:95-113.

Borchardt, D.C. 1995. Optimierung von Methoden zur Züchtung von Zuckerrüben. Dissertation Universität Hohenheim.

Campbell, C.A. and H.H. Janzen. 1995. Effect of tillage on soil organic matter. Farming for a better environment. pp. 9-11.

Cesareni, G., M.A. Muesing and B. Polisky. 1982. Control of ColE1 DNA replication: The *rop* gene product negatively affects transcription from the replication primer promoter. Proc. Natl. Acad. Sci. 79:6313-6317.

CFIA. 2002. Regulatory Directive Dir2002-01: The Biology of *Beta vulgaris* L. (Sugar Beet). Canadian Food Inspection Agency. World Wide Website (http://www.inspection.gc.ca).

Cole, D.J., 1985. *In* The Herbicide Glyphosate. Grossbard, E. and Atkinson, D., ed., Butterworths, Boston, pp. 48-74.

Coruzzi, G., R. Broglie, C. Edwards and N. Chua. 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.

Coupland, D. 1985. *In* The Herbicide Glyphosate: Grossbard, E: Atkinson, D. Eds.; Butterworths, London, pp. 25-34.

CTIC, 2000. Conservation for Agriculture's Future. Top 10 conservation tillage benefits. http://www.ctic.purdue.edu/Core4/CT/CTSurvey/10Benefits.html

Dark, S.O.S. 1971. Experiments on the cross-pollination of sugar beet in the field. J. Natn. Inst. Agric. Bot. 12:242-266.

Della-Cioppa, G., S.C. Bauer, B.K. Klein, D.M. Shah, R.T. Fraley and G.M. Kishore. 1986. Translocation of the precursor of 5-enolpyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants in vitro. Proc. Natl. Acad. Sci. USA 83:6873-6877.

Della-Cioppa, G., S.C. Bauer, M.T. Taylor, D.E. Rochester, B.K. Klein, D.M. Shah, R.T. Fraley and G.M. Kishore. 1987. Targeting a herbicide-resistant enzyme from Escherichia coli to chloroplasts of higher plants. Bio/Technology 5:579-584.

Depicker, A., S. Stachel, P. Dhaese, P. Zambryski and H. Goodman. 1982. Nopaline Synthase: Transcript mapping and DNA sequence. J. Molec. Appl. Genet. 1:561-573.

Desplanque, B., P. Boudry, K. Broomberg, P. Saumitou-Laprade, J. Cuguen and H. Van Dijk. 1998. Genetic diversity and gene flow between wild, cultivated and weedy forms of *Beta vulagris* L. (Chenopodiaceae), assessed by RFLP and microsatellite markers. Theor. Appl. Genet. 8:1194-1201.

Dexter, A.G. 2000. Weed control guide for sugar beet. pp. 3- 38. *In* 1999 Sugar beet research and extension reports, North Dakota State University Extension.

Dexter, A.G., J.L Luecke and L.J. Smith 2000. Influence of cultivation on yield of Roundup Ready and Liberty Link sugarbeet. pp. 100-103. *In* 1999 Sugar beet research and extension reports, North Dakota State University Extension.

Dexter, A.G. and J.L. Luecke. 2000a. Survey of weed control and production practices on sugarbeet in eastern North Dakota and Minnesota - 1999. pp. 39- 68. *In* 1999 Sugar beet research and extension reports, North Dakota State University Extension.

Dexter, A.G. and J.L. Luecke. 2000b. Survey of weed control and production practices on sugarbeet in western North Dakota and eastern Montana - 1999. pp. 69- 89. *In* 1999 Sugar beet research and extension reports, North Dakota State University Extension.

Dexter, A.G. and J.L. Luecke. 2003. Survey of weed control and production practices on sugarbeet in eastern North Dakota and Minnesota - 2002. pp. 35- 64. *In* 2002 Sugarbeet Research and Extension Reports, North Dakota State University Extension, Vol. 33.

Dexter, A.G. and R.K. Zollinger. 2003. Weed Control Guide for Sugarbeet. pp. 3-34. *In* 2002 Sugarbeet Research and Extension Reports, North Dakota State University Extension, Vol. 33.

DLG (Deutsche Landwirtschafts-Gesellschaft). 1991. DLG Futterwerttabelle 6. Auflage, pp. 24 and 44.

Doane. 2003. 2002 AgroTrak. Doane Marketing Research, Inc. Unpublished survey data.

Duke, S.O. 1998. *In* Herbicides: Chemistry, Degradation and Mode of Action; Kearney, P.C. & Kaufman, D.D.Eds., Marcel Decker Inc., New York, 1988, 3:1-70.

Estok, D., B. Freedman and D. Boyle. 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.

FAO. 1999. Sugar Beets / White Sugar. Food and Agriculture Organization of the United Nations, Agribusiness Handbooks, vol. 4, pp. 35-43.

Fawcett, R.S. 1995. Agricultural tillage systems: Impacts on nutrient and pesticide runoff and leaching. Soil and Water Conservation. pp. 48-50.

Fling, M.E., J. Kopf and C. Richards. 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase. Nucl. Acids Res. 13:7095-7106.

Franz, J.E., M.K. Mao and J.A. Sikorski. 1997. Glyphosate Tolerance. pp. 617-642. *In* Glyphosate: A Unique Global Herbicide. ACS Monograph 189, American Chemical Society, Washington, DC.

Geisy, J.P., S. Dodson and K.R. Solomon. 2000. Ecotoxicological risk assessment for Roundup herbicide. Rev. Environ. Contam Toxicol 167:35-120.

Gianessi, L.P., C.S. Silvers, S. Sankula and J.E. Carpenter. 2002. Plant Biotechnology: Current and Potential Impact for Improving Pest Management in U.S. Agriculture an Analysis of 40 Case Studies: Herbicide Tolerant Sugarbeet. National Center for Food and Agricultural Policy, Case 20, pp. 2-21.

Gowda, S., F.C. Wu and R.J. Sheperd. 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.

Gustafson, D.I. 1989. Groundwater ubiquity score: A simple method for assessing pesticide leachability. Environmental Toxicology and Chemistry 8:339-357.

Hanson, L. 2003. Effect of treating sugar beet with the herbicide glyphosate on *Fusarium* yellows severity. Western Society of Crop Science Meeting, Fort Collins, Co. June.

Harrison, L., M. Bailey, M. Naylor, J. Ream, B. Hammond, D. Nida, B. Burnette, T. Nickson, T. Mitsky, M. Taylor, R. Fuchs and S. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. American Institute of Nutrition, Vol. 126, No. 3, pp. 728-740.

Haslam, E. 1974. The Shikimate Pathway, John Wiley and Sons, New York, New York.

Heap, I. The International Survey of Herbicide Resistant Weeds. Online. Internet. June 28, 2002. www.weedscience.com.

Herrmann, K.M. 1983. The common aromatic biosynthetic pathway. *In* Amino Acids: Biosynthesis and Genetic Regulation. K.M. Herrmann and R.L. Somerville (eds.), Addison-Wesley, Reading, MA. pp. 301-322.

Holt, J.S. and H. M. Le Baron. 1990. Significance and distribution of herbicide resistance. Weed Technology 4:141-149.

Howard, E., V. Citovsky and P. Zambryski. 1990. The T-complex of Agrobacterium tumefaciens. *In* Plant Gene Transfer. C.J. Lamb and R. N. Beachy (eds.), Alan R. Liss, Inc., New York, New York. pp. 1-12.

James, C. 2001. Global Review of Commercialized Transgenic Crops: 2000. ISAAA Briefs No. 23. ISAAA: Ithaca, New York.

Klee, H.J., Y.M. Muskopf and C.S. Gasser. 1987. Cloning of an Arabidopsis thaliana gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. Mol. Gen. Genet. 210:437-442.

Kniss, A. R., R.G. Wilson, A.R. Martin, P.A. Burgener and D.M. Feuz. 2003. Economic

evaluation of glyphosate-resistant and conventional sugarbeet (*Beta vulgaris*). Accepted for press in Weed Technology, July 2003.

Kremer R.J., P.A. Donald, A.J. Keaster and H.C. Minor. 2000. Glyphosate-tolerant soybean affects root and soil Fusarium and soybean cyst nematode. Proceedings of the North Central Weed Science Society. 55:68.

Le Baron, H. M. 1991. Herbicide resistant weeds continue to spread. Resistant Pest Management Newsletter 3:36-37.

Lee C.D., D. Penner and R. Hammerschimidt. 2000. Influence of formulated glyphosate and activator adjuvants on *Sclerotinia sclerotiorum* in glyphosate-resistant and -susceptible Glycine max. Weed Science 48:710-715.

Lightfoot, D. 2002. Roundup Ultra on Roundup Ready soybean: effects on root infection by *Fusarium solani* f. sp. glycines and sudden death syndrome. (submitted).

Lüdecke, H. and I. Feyerabend. 1958. Beiträge zum Saponingehalt der Zuckerrübe. Zucker 17:389-393.

Malik, J., G. Barry and G. Kishore. 1989. The Herbicide Glyphosate. BioFactors. 2:17-25.

Märländer, B., H. Glattkowski and K. Buchholz. 1996. Entwicklung einer Formel zur Bewertung der Technischen Qualität der Zuckerrüben in Deutschland. IIRB 59th Congress, Brussels. pp. 343-352.

McGinnis, R.A. 1982. Beet – Sugar Technology. Beet Sugar Development Foundation, C&M Press, Denver, CO.

Mesbah, A., S.D. Miller, K.J. Fornstrom and D.E. Legg. 1994. Sugar Beet – Weed Interactions. University of Wyoming, Agricultural Experiment Station, publication B-998.

Morelli, G., F. Nagy, R.T. Fraley, S.G. Rogers and N.H. Chua. 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.

Mountain, A. 1989. Gene expression systems for Bacillus subtilis. pp. 73-114. *In* Bacillus. Vol. 2 of Biotechnology Handbooks, Plenum Press, New York, New York.

OECD. 1999. 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). Series on Harmonization of Regulatory oversight in Biotechnology No. 18. Environment Directorate/ Joint meeting of the chemicals committee and the working party on chemicals. Organization for Economic Co-Operation and Development, Paris, France.

Oakenfull, D. and G.S. Sidhu. 1989. Saponins. pp. 97-141. *In* Toxicants of Plant Origin. Vol. II Glycosides. CRC Press, Boca Raton, Florida.

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

Padgette, S.R., D.B. Re, G.F. Barry, D.E. Eichholtz, X. Delannay, R.L. Fuchs, G.M. Kishore and R.T. Fraley. 1996. New weed control opportunities: Development of soybeans with a Roundup Ready Gene. CRC Press Inc. 4:54-84.

Pratley, J., B. Baines, P. Eberbach, M. Incerti and J. Broster. 1996. Glyphosate resistance in annual ryegrass. Proc. Eleventh Ann. Conf. Grassld. Soc. NSW. 122.

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

Ridout, C.L., K.R. Price, G. Parkin, M.G. Dijoux and C. Lavaud. 1994. Saponins from sugar beet and the floc problem. J. Agric. Food Chem. 42:279-282.

Rogers, S.G., H. Klee, R. Horsch and R.T. Fraley. 1987. Improved vectors for plant transformation: expression cassette vectors and new selectable markers. Meth. Enzymol. 153:253-277.

Saiki, R.K. 1990. Amplification of genomic DNA, p. 13-20. *In* PCR Protocols: A Guide to Methods and Applications. M.A. Innis, D. Gelfand, J.J. Sninsky, and T.J. White (ed) Academic Press, San Diego.

Sanger, M., S. Daubert and R.M. Goodman. 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 Mol. Biol. 14:433-443.

Sanogo, S., X.B. Yang and P. Lundeen. 2001. Does large-scale cropping of herbicide-resistant cultivars increase the incidence of polyphagous soil-borne plant pathogens? Plant Disease 85:773-779.

Shah, D.M., R. Horsch, H. Klee, G. Kishore, J. Winter, N. Tumer, C. Hironaka, P. Sanders, C. Gasser, S. Aykent, N. Siegel, S. Rogers and R.T. Fraley. 1986. Engineering herbicide tolerance in transgenic plants. Science 233: 478-481.

Shaner, D.L. 1995. Herbicide Resistance: Where are we? How did we get here? Where are we going? Weed Technology 9:850-856.

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

Smed, E., A.W. Huijbregts, D. Hadjiantoniou and G. Vallini. 1996. Influence of agronomic factors on nonconventional parameters of the internal quality of sugarbeet. IIRB 59th Congress, Brussels. pp 385-389.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Steen, R.A. 2000. Results of American Crystal's 1999 official coded variety trials. pp. 287-334. *In* 1999 Sugar beet research and extension reports, North Dakota State University Extension. Vol. 30, January 2000.

Sutcliffe, J.G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symposium 43:77-90.

Termorshuizen, A.J. and L.A.P. Lotz. 2002. Does large-scale cropping of herbicide-resistant cultivars increase the incidence of polyphagous soil-borne plant pathogens? Outlook on Agriculture 31:51-54.

Timko, M. P., L. Herdies, E. de Alameida, A. R. Cashmore, J. Leemans and E. Krebbers. 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, and R. M. Ottenbrite, editors. ACS Books, Washington, DC.

USDA-FAS. 2002. United States Department of Agriculture, Foreign Agricultural Service. World Wide Website (http://www.fas.usda.gov).

USDA-NASS. 2001. Agricultural Chemical Usage: 2000 Field Crops Summary. United States Department of Agriculture, National Agricultural Statistics Service, Washington, DC. Ag Ch 1 (01) b, May.

USDA-NASS. 2002. Acreage: 2002 Summary. United States Department of Agriculture, National Agricultural Statistics Service, Agricultural Statistics Board, Washington, DC. Cr Pr 2-5 (6-02), June.

USDA-NASS. 2003a. Crop Values: 2002 Summary. United States Department of Agriculture, National Agricultural Statistics Service, Agricultural Statistics Board, Washington, DC. Pr 2 (03), February.

USDA-NASS. 2003b. Prospective Plantings: 2003 Summary. United States Department of Agriculture, National Agricultural Statistics Service, Agricultural Statistics Board, Washington, DC. CR Pr 2-4 (3-03), March.

Van Gessel, M.J., 2001. Glyphosate resistant horseweed from Delaware. Weed Science 49:703-705.

Wan, M.T., J.E. Rahe and R.G. Watts. 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.

White, F.F. 1989. Vectors for gene transfer in higher plants. pp. 3-34. *In* Plant Biotechnology. S. Kung and C.J. Arntzen (eds.), Butterworths, Boston, Massachusetts.

Williams, G.M., R. Kroes and I. Munro. 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate for humans. pp. 117-165. *In* Regulatory Toxicology and Pharmacology, Vol. 31.

Wilson, R.G. 2001. Sugarbeet Production Guide. University of Nebraska Cooperative Extension publication EC01-156.

Wilson, R.G., D.C. Yonts and J.A. Smith. 2002. Influence of glyphosate and glufosinate on weed control and sugarbeet (*Beta vulgaris*) yield in herbicide-tolerant sugarbeet. Weed Technology 16:66-73.

Wilson, R.G. and P. Stahlman. 2003. Maintain the rate. Pp. 12-13, Nebraska Farmer, June edition.

WSSA, 1994. Herbicide Handbook, 7th edition. Ed. William H. Ahrens. Weed Society of America, 1508 West University Avenue. Champaign, Illinois. ISBN# 0-911733-18-3.

Yang, X., S.K. Harrison and R.M. Riedel. 2002. Soybean (Glycine max) response to glyphosate and soybean cyst nematode (Heterodera glycines). Weed Technol. 16:332-339.

Zollinger, R.K. 2000. North Dakota Weed Control Guide. NDSU Extension Service (copy available on Internet:
http://www.ext.nodak.edu/extpubs/plantsci/weeds/w253/w253w.htm

APPENDIX 1

APPEARANCE OF GLYPHOSATE-RESISTANT WEEDS

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. The potential for weeds to become resistant to glyphosate is important to Monsanto because resistance can adversely impact the utility and life cycle of our products if it is not managed properly. The risk of weeds developing resistance and the potential impact on the usefulness of an herbicide vary greatly across different modes of action and are dependent on a combination of factors. As leaders in the development and stewardship of glyphosate products for almost thirty years, Monsanto has invested considerably in research to understand the proper uses and stewardship of the glyphosate molecule. This research includes an evaluation of some of the factors that can contribute to the development of weed resistance.

A. The Herbicide Glyphosate

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #'s 1071-83-6 and 38641-94-0), the active ingredient in the Roundup family of nonselective, foliar-applied, broad-spectrum, post-emergent herbicides (Baird, 1971; Malik et al., 1989), is the world's most popular herbicide active ingredient. Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate kills plants cells by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinrücken and Amrhein, 1980). The aromatic amino acid pathway is not present in mammalian metabolic pathways (Cole, 1985). This contributes to the selective action of glyphosate toward plants but not mammals. Glyphosate has favorable environmental and safety characteristics, such as rapid soil binding (resistance to leaching) and biodegradation (which decreases persistence), as well as extremely low toxicity to mammals, birds and fish (Malik et al., 1989). Glyphosate is classified by the EPA as Category E (evidence of noncarcinogenicity for humans) (57 FR 8739).

B. Characteristics Related to Resistance

Today, some 169 herbicide-resistant species and 282 biotypes within those species have been identified (Heap, 2003). Most of these are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Le Baron, 1991; Shaner, 1995). Resistance usually has developed because of the long residual activity of these herbicides with the capability to control weeds over a long period and the selection pressure exerted by the repeated use of herbicides with a single target site and a specific mode of action. Using these criteria, and based on current use data, glyphosate is considered to be a herbicide with a low risk for weed resistance (Benbrook, 1991). Nonetheless, a question has been raised as to whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide.

It is important to recognize that weed resistance is a herbicide-related issue, not a crop-

related issue. The use of a specific herbicide with a herbicide tolerant crop is no different than the use of a selective herbicide in a conventional crop from a weed resistance standpoint. While the incidence of weed resistance is often associated with repeated applications of a herbicide product, its development depends very much on the specific herbicide chemistry in question as well as the plant's ability to inactivate it. Some herbicide products are much more prone to develop herbicide resistance than others. Glyphosate has been used extensively for three decades with very few cases of resistance development, particularly in relation to many other herbicides. This is largely due to many unique properties of glyphosate that make the development of resistance unlikely, including highly-specific target sites in the plant, limited metabolism in plants, and a lack of soil residual activity. A summary of those factors is provided below.

B.1. Target site specificity

Target site alteration is a common resistance mechanism among many herbicide classes, such as acetolactate synthase (ALS) inhibitors and triazines, but is less likely for glyphosate.

A herbicide's mode of action is classified by the interference of a critical metabolic process in the plant by binding to a target protein and disrupting the required function. The "specificity" of this interaction is critical for the opportunity to develop target site-mediated resistance. Because the herbicide comes into contact with discreet amino acids during protein binding, changing one of these contact point amino acids can interrupt this binding.

The specificity of inhibitor binding is dependent on the number and type of the amino acids serving as contact points and can be measured indirectly by counting the number of unique compounds that can bind to the same site. On one extreme, the only herbicide compound known to bind to EPSPS is glyphosate, demonstrating that the binding is highly specific. Single amino acid substitutions near the active site that can make glyphosate binding slightly weaker have been observed; however, these enzymes are also less fit. Similarly, high specificity is observed for glutamine synthetase, binding three compounds including phosphinothricin in the active site (Crespo et al., 1999). Paraquat and diquat are the only two herbicides inhibiting photosystem I. No target site mutations have been reported to be responsible for resistance in these systems (Powles and Holtum, 1994).

On the other extreme are target enzymes that are efficiently inhibited by a wide array of compounds, e.g., ALS is inhibited by 53 separate herbicide compounds and acetyl CoA carboxylase (ACCase) is inhibited by 21 separate herbicide compounds that bind both within and outside the active site (HRAC, 2002; Tranel and Wright, 2002). These cases demonstrate that numerous non-critical amino acids are involved outside of the active site, offering a relatively large range of permissible mutations. In these two cases, a single amino acid change can result in virtual immunity to these classes of herbicides and has directly led to the preponderance of resistant weed species for these mode-of-actions (MOAs), 79 and 30, respectively.

Glyphosate competes for the binding site of the second substrate, phosphoenolpyruvate (PEP), in the active site of EPSPS and is a transition state inhibitor of the reaction (Steinrücken and Amrhein, 1984). This was recently verified by x-ray crystal structure (Schonbrunn et al., 2001). As a transition state inhibitor, glyphosate binds only to the key catalytic residues in the active site. Catalytic residues are critical for function and cannot be changed without a lethal or serious fitness penalty. Furthermore, very few selective changes can occur near the active site of the enzyme to alter the competitiveness of glyphosate without interfering with normal catalytic function. Therefore, target site resistance is highly unlikely for glyphosate. This was further illustrated in that laboratory selection for glyphosate resistance using whole plant or cell/tissue culture techniques were unsuccessful (Jander et al., 2003; Widholm et al., 2001; OECD, 1999).

B.2. Metabolism in plants Metabolism of the herbicide active moiety is often a principle mechanism for the development of herbicide resistance. The lack of glyphosate metabolism or significantly slow glyphosate metabolism has been reported in multiple plant species and reviewed in various publications (Duke, 1988; Coupland, 1985). Therefore, this mechanism is unlikely to confer resistance to glyphosate in plants.

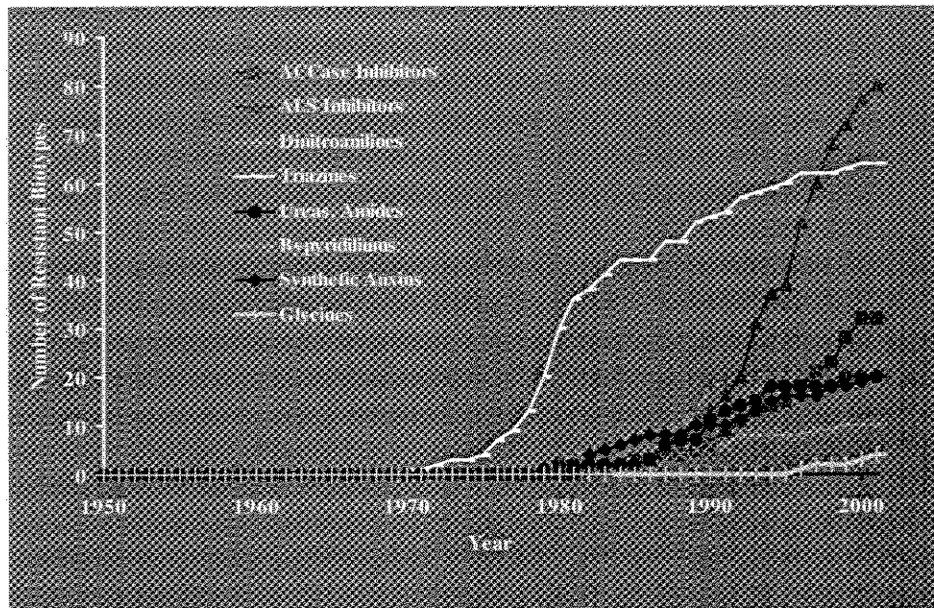
B.3. Soil residual activity

Herbicides with soil residual activity dissipate over time in the soil, resulting in a sublethal exposure and low dose selection pressure over a period of time. Glyphosate adsorption to soils occurs rapidly, usually within one hour (Franz et al., 1997). Soil-bound glyphosate is therefore unavailable to plant roots, so the impact of sublethal doses over time is eliminated. The fact that glyphosate is only active foliarly allows for the use of a high dose weed management strategy.

The graph in Figure 1 illustrates the occurance of weed resistance over time to various herbicide families. The different slopes observed are largely due to the factors described above, which relate to chemistry and function, in addition to levels of exposure in the field. Glyphosate is a member of the glycine family of herbicides, which has experienced very limited cases of resistance despite almost three decades of use. On the other hand, numerous weed species have developed resistance to the ALS inhibitors and triazine families even after they were available for only a relatively short period of time (Heap, 2003).

It is also important to recognize that each herbicide targets a large number of weeds, so the development of resistance in certain species does not mean the herbicide is no longer useful to the grower for control of other species. For example, resistance of certain weeds to imidazolinone and sulfonylurea chemistries developed within three to five years of their introduction into cropping systems. Nevertheless, Pursuit (imidazolinone) herbicide maintained a 60% share of the U.S. soybean herbicide market despite the presence of a large number of resistant weeds because it was used in combination with other herbicides that controlled the resistant species. How weed resistance impacts the use of a particular herbicide varies greatly depending on the herbicide chemistry, the biology of the weed, the availability of other control practices and the diligence with which it is managed.

Figure 1. Number of Herbicide Resistant Weed Species Found Over Time



C. Weeds Resistant to Glyphosate

Weed resistance is generally defined as the naturally occurring inheritable ability of some weed biotypes within a given weed population to survive a herbicide treatment that should, under normal use conditions, effectively control that weed population. Thus, a resistant weed must demonstrate two criteria: 1) the ability to survive application rates of a herbicide product that once were effective in controlling it; and 2) the ability to pass the resistance trait to seeds. Procedures to confirm resistance generally require both field and greenhouse analyses, particularly if the level of resistance is relatively low as is generally seen for cases of glyphosate resistance.

As part of our current product stewardship and customer service policy, Monsanto investigates cases of unsatisfactory weed control to determine the cause, as described in the performance evaluation program outlined in section E of this Appendix. Weed control failures following application of Roundup agricultural herbicides are most often the result of management and/or environmental issues and are very rarely the result of herbicide resistance. The procedures included in Monsanto's performance evaluation program provide early detection of potential resistance, field and greenhouse protocols to investigate suspected cases and mitigation procedures to respond to confirmed cases of glyphosate resistance.

To date, biotypes of only four weed species resistant to glyphosate have been identified. In all cases, Monsanto worked with local scientists to identify alternative control options that have been effective in managing the resistant biotype.

C.1. *Lolium rigidum*

In 1996 in Australia, it was reported that a biotype of annual ryegrass (*Lolium rigidum*) was surviving application of recommended rates of glyphosate (Pratley et al., 1996). A collaboration was established with Charles Sturt University to develop an agronomic understanding of the biotype and investigate the mechanism of resistance. Where the biotype has been found, it has occurred within isolated patches within a field and does not appear to be widespread. The resistant biotype is easily controlled within conservation and conventional tillage systems with other herbicides, tillage or seed removal.

A large body of biochemical and molecular biology experiments between Australian ryegrass biotypes resistant and susceptible to glyphosate indicate that the observed resistance is due to a combination of factors. The mechanism of resistance appears to be multigenic and caused by a complex inheritance pattern, which is unlikely to occur across a wide range of other species. The mechanism is yet to be fully defined despite significant research effort; however, reduced cellular transport of glyphosate has been proposed (Lorraine-Colwill et al., 2003).

The resistant annual ryegrass biotype has also been observed in orchard systems of California and South Africa. Similar to the Australian locations, these fields are small and isolated. Monsanto established collaborations with local scientists to identify alternative control mechanisms, and the use of other herbicides, tillage, mowing and seed removal have been very effective in controlling the ryegrass.

Annual ryegrass has not been identified as a significant weed concern in primary sugar beet production areas.

C.2. *Lolium multiflorum*

A population of Italian ryegrass (*Lolium multiflorum*) was reported to survive labeled rates of glyphosate by a scientist conducting greenhouse and field trials in Chile. Monsanto conducted field and greenhouse trials to confirm the resistance and worked with the researcher to identify alternative control options. The resistant biotype has been found on a few farms and is easily controlled through tank mixes with other herbicides and cultural practices.

Similar to annual ryegrass, Italian ryegrass has not been identified as a significant weed concern in primary sugar beet production areas.

C.3. *Eleusine indica*

A population of *Eleusine indica (*goosegrass*)* was reported to survive labeled rates of glyphosate in some orchard systems in Malaysia. Monsanto entered into collaborations with the Universiti Kebangsaan Malaysia (National University of Malaysia) and identified alternative control options to effectively manage the resistant biotype. Extensive molecular investigations determined that some of the resistant goosegrass plants have a modified EPSPS that is 2-4 times less sensitive to glyphosate than more sensitive biotypes (Baerson et al., 2002). Partial sequencing of the EPSPS synthase gene in the R biotype of resistant goosegrass confirmed that a mutation has occurred, where there has been a substitution of proline with serine or threonine at amino acid 106 (Ng et al., 2003). This mutation may account for these resistant goosegrass plants that are less sensitive to glyphosate. However, some individuals did not exhibit the enzyme modification, suggesting that different mechanisms may be at play or resistance may be due to a combination of factors.

The resistant biotypes are easily controlled through application timing (applying glyphosate during the early growth stages), use of other herbicides, tillage and other cultural control practices.

Goosegrass is a warm season annual grass that has a low, “creeping” growth habit. It is not a weed problem in sugar beet production.

C.4. *Conyza Canadensis*

Laboratory and field investigations confirmed the presence of a glyphosate-resistant biotype of marestail (*Conyza canadensis*) in certain states of the eastern and southern U.S. (VanGessel, 2001). The mechanism of resistance in the marestail biotype is currently under investigation. Findings from initial studies were presented at national and regional U.S. Weed Science Society meetings in 2001 and 2002 (Heck et al., 2002), and subsequent data will be presented and/or published for review by the scientific community.

Investigations thus far indicate that this biotype has a heritable resistance ranging up to approximately 8-10X field herbicide application rates. Data to date indicate that the inheritance is semi-dominant and transmissible through both gametes. The metabolism of glyphosate is not a mechanism contributing to the resistance observed. Our results demonstrate a strong correlation between impaired glyphosate translocation and resistance. Tissues from both S and R biotypes showed elevated levels of shikimate, suggesting that EPSPS remained sensitive to glyphosate. Analysis of tissue shikimate levels relative to those of glyphosate demonstrated a reduced efficiency in EPSPS inhibition in the R biotypes. Our results are consistent with two potential mechanisms of glyphosate resistance – impaired translocation and reduced EPSPS inhibition. Our current working hypothesis is that marestail resistance results from an alteration of glyphosate distribution in cellular compartments that impairs its phloem loading and plastidic import.

The resistant biotype has been observed in conventional and Roundup Ready cotton and soybean fields. As in other cases, Monsanto responded to weed control inquiries and alternative weed control options were provided. Growers in and around the areas where the biotype has been detected are advised to utilize tank-mix treatments which have proven effective.

Marestail can be found in sugar beet fields in the U.S. but is not considered a weed of concern. The resistant biotype has not been observed in the northern and western states where sugar beets are grown, and a stewardship program is in place to minimize movement of the resistant biotype beyond the southern and eastern regions where it has been observed. Marestail competes best in undisturbed areas and can be easily managed by sugar beet growers through tillage practices commonly used to prepare their fields for planting.

In summary, Monsanto has effective product stewardship and customer service practices established to directly work with the grower communities and provide appropriate control measures for glyphosate-resistant weeds. Monsanto has collaborated with academic institutions to study these glyphosate-resistant biotypes and findings have been communicated to the scientific community through publications in peer-reviewed scientific journals and scientific meetings.

D. Weed Management Strategies for Glyphosate

A key element of good weed management is using the correct rate of glyphosate at the appropriate window of application for the weed species and size present. Higher herbicide doses result in higher weed mortality and lower frequency of resistance genes in the surviving population (Matthews, 1994). However, low herbicide rates may allow both heterozygous and homozygous resistant individuals to survive (Maxwell and Mortimer, 1994), further contributing to the build up of resistant alleles in a population. As resistance is dependent upon the accumulation of relatively weak genes, which appears to be the case for one or more of the four weed species that have developed resistance to glyphosate, using a lethal dose of herbicide is critical.

Results that support these strategies are beginning to emerge from recent field research studies at several universities (Roush et al., 1990). Various weed management programs have been evaluated since 1998 to determine how they impact weed population dynamics. Studies were initiated in Colorado, Kansas, Nebraska, Wyoming (Wilson and Stahlman, 2003), and Wisconsin (Stoltenburg, 2002) to evaluate the continuous use of Roundup Ready technology with exclusive use of glyphosate or inclusion of herbicides with other modes of action, and rotation away from Roundup Ready technology. These treatment regimes were compared to a conventional herbicide program for each crop evaluated. General observations after five years are:

1. Use of a continuous Roundup Ready cropping system with either glyphosate alone at labeled rates or incorporation of herbicides with other modes of action resulted in excellent weed control with no weed shifts or resistance reported.

2. Use of glyphosate at below labeled rates resulted in a weed shift to common lambsquarters at two locations (Nebraska and Wyoming).
3. In Wisconsin, ALS-resistant giant ragweed was selected for in the broad-spectrum residual herbicide regime implemented in the conventional corn cropping system. The continuous glyphosate system (using labeled rates) resulted in no significant weed shifts.

By using glyphosate at the recommended lethal dose, the buildup of weeds with greater inherent tolerance or any potential resistance alleles has been avoided over the duration of these studies. These results indicate that continuous Roundup Ready systems used over five years did not create weed shifts or resistant weeds when the correct rate of glyphosate was applied and good weed management was practiced.

E. Glyphosate Stewardship Program

Commercial experience, field trials and laboratory research demonstrate that one of the most important stewardship practices is achieving maximum control of the weeds. This can be accomplished by using the correct rate of glyphosate at the appropriate window of application for the weed species and size present, and using other tools or practices as necessary.

As the recognized leader in the development and commercialization of glyphosate, Monsanto is committed to the proper use and long-term effectiveness of glyphosate through a four-part stewardship program: developing appropriate weed control recommendations; conducting research to refine and update recommendations; educating growers on the importance of good weed management practices; and responding to repeated weed control inquiries through a performance evaluation program.

E.1. Development of local weed management recommendations to ensure maximum practical control is achieved

Weed control recommendations in product labels and informational materials are based on local needs to promote the use of the management tool(s) that are most appropriate technically and economically for each region. Furthermore, growers are instructed to apply the same principles when making weed control decisions for their own farm operation. Multiple agronomic factors, including weed spectrum and population size, application rate and timing, herbicide resistance status (where applicable) and an assessment of past and current farming practices used in the region or on the specific operation are considered to ensure appropriate recommendations for the use of glyphosate to provide effective weed control. Carefully developing and regularly updating the use recommendations for glyphosate are fundamental to Monsanto's stewardship program.

Weed spectrum: Weed spectrum refers to all of the weed species present in a grower's field and the surrounding areas that may impact those fields. The spectrum may vary across regions, farm operations, and even among fields within a farm operation depending on environmental conditions and other factors. Weed control programs should be tailored

on a case-by-case basis by identifying the target weeds present, considering the efficacy of glyphosate and other weed management tools against those particular weeds, and assessing if any are unlikely to be controlled sufficiently with glyphosate alone, i.e., the weeds are not included on the label, are difficult to control based on agronomic and/or environmental conditions, or have documented resistance to glyphosate. Specific formulations, rates, application parameters, and additional control tools are recommended as necessary to optimize control of all weeds in that system.

Application Rate: Application rate is integral to the correct use of glyphosate and critical to obtain effective weed control. Significant research has been conducted to identify the appropriate rate of glyphosate required for a particular weed at various growth stages in various agronomic and environmental conditions. These rates are included in rate tables provided in product labels and other materials. In addition, Monsanto recommends that growers use the rate necessary to target the most difficult to control weed in the field to minimize weed escapes. When using tank mixes, growers should consider the potential impacts on glyphosate efficacy through antagonism or below-recommended rates and make adjustments accordingly.

Application Timing: Application timing is based on the growth stage of weeds, the size/biomass of weeds and the agronomic and environmental conditions at the time of application. Delaying the application of glyphosate and allowing weeds to grow too large before applying the "recommended rate" of glyphosate will result in poor efficacy. Applying glyphosate at a time when weeds are under agronomic stress (e.g., insect /disease pressure) or environmental stress (e.g., moisture/drought/cold condition) can also result in poor weed control.

Compensating for a delayed application through subsequent applications may not be effective, as the first application may inhibit weed growth and impair the efficacy of the second application because weeds may not be in an active growth process.

Correct application timing is dependent on the combined management of the weed spectrum, the size and layout of the farm operation and the feasibility to make timely applications of all weeds in the fields with the labor and equipment available. Monsanto recommends an application timeline that targets susceptible growth stages of all weeds, and, where applicable, includes recommendations for inclusion of additional control tools as necessary to optimize control of all weeds on that farm.

Finally, it is important to assess the current agronomic practices used in a particular region or farm operation to integrate the glyphosate recommendations into the grower's preferred management system. Variables such as tillage methods, crop rotations, other herbicide programs, other agronomic practices and the resistance status of the weeds to herbicides other than glyphosate can impact the spectrum of weeds present and the tools available to the grower.

Weed management recommendations communicated to growers also incorporate other

components of the glyphosate stewardship program including use of high quality seed, employing sanitary practices such as cleaning equipment between fields, and scouting fields and reporting instances of unsatisfactory weed control for follow up investigation.

E.2. Research

A fundamental component of Monsanto's leadership in glyphosate stewardship is research on the recommended use of glyphosate and factors impacting its effectiveness. In addition to extensive analyses conducted to determine the correct application rate of glyphosate prior to product registration, ongoing agronomic evaluations are conducted at the local level to refine weed management recommendations for specific weed species in specific locations.

Weed efficacy trials are part of ongoing efforts by Monsanto to tailor recommendations to fit local conditions and grower needs. Application rate and timing, additional control tools and other factors are included in these analyses. As a result of weed efficacy trials, changes are made to specific weed control recommendations where and when applicable, and modifications to local recommendations are communicated to growers through informational sheets and other methods.

E.3. Education and communication efforts

Another key element of effective product stewardship and appropriate product use is education to ensure that growers understand and implement effective weed management plans and recommendations. Monsanto communicates weed management recommendations through multiple channels and materials to multiple audiences.

All Monsanto technical and sales field representatives are required to take a weed management training course to understand the glyphosate stewardship program and the importance of proper product use. The training program is supported by ongoing weed management updates that highlight seasonal conditions and recommendations.

Monsanto weed management recommendations and the importance of sound agronomic practices are communicated to growers, dealers and retailers, academic extension agents and crop consultants through multiple tools:

1. Technology training programs: Highlighting weed management principles, weed management plans and practical management guidelines.
2. Technology use guide: Includes tables outlining appropriate rate and timing for different weed species and sizes.
3. Grower meetings: Conducted prior to planting to emphasize the importance of following local application recommendations.
4. Marketing programs: Designed to reinforce and encourage the continued adoption and use of weed management recommendations by the grower (e.g., recommended rate and timing of application, and additional weed control tools when applicable).

5. Informational Sheets: Issued to growers and dealers/retailers to highlight local recommendations for specific weeds.

As with most stewardship efforts, education is key to help growers and other stakeholders understand the importance of proper product use and encourage those practices in the field.

E.4. Performance inquiry evaluation and weed resistance management plan

To support and enhance Monsanto's weed management principles and recommendations, Monsanto has implemented a performance evaluation program based on grower performance inquiries and field trial observations. The goal of the program is to adapt, modify and improve Monsanto's weed control recommendations, with a focus on:

1. Particular weeds and growing conditions,
2. Providing product support to customers who are not satisfied with their level of weed control, and
3. Identifying and investigating potential cases of glyphosate resistance early so that mitigation strategies can be implemented.

The grower generally reports instances of unsatisfactory weed control following a glyphosate application to either Monsanto or the retailer. Monsanto investigates these inquiries immediately, as it is important to maintain the customer's satisfaction and is part of the stewardship committment.

The vast majority of inquiries are due to application error or environmental conditions at the time of herbicide application. A system is in place to investigate a repeated performance inquiry for a specific weed within a specific field that occurs within the same growing season. The investigation considers the various factors that could account for ineffective weed control such as (but not limited to):

1. Application rate and timing,
2. Weed size and growth stage,
3. Application equipment set up and calibration, and
4. Environmental and agronomic conditions at time of application.

In all cases, the first priority is to provide control options to the grower so that satisfactory weed control is achieved for that growing season. The majority of repeated product performance inquiries are due to improper application or environmental /agronomic conditions and, when properly addressed, are not repeated. However, if unsatisfactory weed control occurs again in that field and does not appear to be due to application or growing condition factors, then steps are taken to determine whether resistance is the cause, as outlined in the Monsanto Weed Resistance Management Plan.

The Monsanto Weed Resistance Management Plan consists of three elements:

1. Identification process for potential cases of glyphosate resistance,
2. Initiation of steps to respond to cases of suspected resistance, and
3. Development and communication of guidelines to incorporate resistance mitigation into weed management recommendations.

Identification of potential cases of glyphosate resistance is accomplished through evaluation of product performance inquiries and local field trials. These efforts provide an early indication of ineffective weed control that may indicate potential resistance.

If the follow up investigation clearly indicates that the observation is due to application error or agronomic/environmental conditions, then appropriate control options are recommended to the grower for that season and the grower receives increased education on the importance of proper product use. The vast majority of weed control inquiries fall into this category.

If repeated lack of control is observed and does not appear to be due to application error or environmental conditions, then a field investigation is conducted by Monsanto to analyze control of the weed more thoroughly.

Weeds must be actively growing in order for glyphosate to be effective. Application error or environmental conditions that result in insufficient glyphosate to kill the weed often stunt its growth such that subsequent applications by the grower are ineffective. Monsanto's field investigations at this stage remove that artifact by ensuring that the weeds tested are in an active growth phase. The vast majority of field investigations do not repeat the insufficient control reported by the grower. If the field investigation confirms that agronomic factors accounted for the observation, then the grower receives increased education on proper application recommendations.

In addition, the internal network of Monsanto technical managers and sales representatives in the surrounding area are notified to highlight any problematic environmental conditions or application practices that may be common in that area. Critical information regarding location, weed species, weed size, rate used and the potential reason for lack of control is documented, and the information is reviewed annually by the appropriate technical manager to identify any trends or learnings that need to be incorporated into the weed management recommendations.

If the reported observation is repeated in the field investigation, then a detailed performance inquiry is conducted and greenhouse trials are initiated. If greenhouse trials do not repeat the observation and the weed is clearly controlled at label rates, then a thorough follow up visit is conducted with the grower to review the application recommendations and conditions of his operation that may be impacting weed control. Monsanto's internal network of agronomic managers is notified of the results to raise awareness of performance inquiries on that weed the following season. If the greenhouse efficacy trials do indicate insufficient control at label rates, then detailed studies are

conducted to determine if the weed is resistant.

Resistance is considered to be confirmed if the following two criteria are deemed to be fulfilled either through greenhouse and field data or experience with similar cases:

1. The suspect plant is demonstrated to tolerate labeled rates of glyphosate that previously were effective in controlling it, and
2. The suspect plant is capable of passing that ability to offspring (i.e., the trait is heritable).

Additional field trials generally are initiated simultaneously as these investigations are conducted to identify the most effective and efficient alternative control options for that weed in various growing conditions. The research may be conducted internally by Monsanto as well as through collaboration with external researchers.

If resistance is confirmed, then the scientific and grower communities are notified as appropriate and a weed resistance mitigation plan is implemented. The mitigation plan is designed to manage the resistant biotype through effective and economical weed management recommendations implemented by the grower. The scope and level of intensity of the mitigation plan will vary depending on a combination of the following factors:

1. Biology and field characteristics of the weed species (seed shed, seed dormancy, etc.),
2. Importance of the weed species in the agricultural system,
3. Resistance status of the weed species to other herbicides with alternate modes of action, and
4. Availability of alternative control options.

These factors are analyzed in combination with economic and practical management considerations to develop a tailored mitigation strategy that is technically appropriate for the particular weed and incorporates practical management strategies that can be implemented by the grower.

Once developed, the mitigation plan is communicated to the grower community through supplemental labeling, informational fact sheets, retailer training programs, agriculture media or other means, as appropriate.

The final step of the Weed Resistance Management Plan may include extensive genetic, biochemical or physiological analyses of confirmed cases of glyphosate resistance in order to elucidate the mechanism of resistance. Findings of this research are communicated to the scientific community through scientific meetings and publications, and information pertinent to field applications is incorporated into weed management recommendations.

F. Summary

Development of weed resistance is a complex process that is very difficult to accurately predict, and no single agronomic practice will mitigate resistance for all herbicides or all weeds. As a result, weed resistance must be managed on a case-by-case basis and management programs need to be tailored to the particular herbicide and grower needs. Using good weed management principles built upon achieving high levels of control through proper application rate, choice of cultural practices and appropriate companion weed control tools will allow glyphosate to continue to be used effectively.

The key principles for effective stewardship of glyphosate use, including use in Roundup Ready crops, are: 1) basing recommendations on local needs and using the tools necessary to optimize weed control; 2) proper rate and timing of herbicide application; and 3) responding rapidly to instances of unsatisfactory weed control.

G. References

Baerson, S.R., D.J. Rodriguez, M. Tran, Y. Feng, N. Biest, and G.M. Dill. 2002. Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiology 129:1265-1275.

Baird, D.D., R.P. Upchurch, W.B. Homesley, and J.E. Franz. 1971. Introduction of a new broad-spectrum post emergence herbicide class with utility for herbaceous perennial weed control. Proceedings of the North Central Weed Control Conference. Dec. 7 –9, 1971. Kansas City, MO. Vol.26:64-68.

Benbrook, C. 1991. Racing against the clock. agrichemical age, pp. 32-33.

Cole, D.J. 1985. *Mode of action of glyphosate – a literature analysis. In* The Herbicide Glyphosate. E. Grossbard and A. Atkinson, Ed. Butterworth's & Co. Boston, 1985. pp. 48-75.

Coupland, D. 1985. *In* The Herbicide Glyphosate; Grossbard, E: Atkinson, D. (Eds.), Butterworths, London, pp. 25-34.

Crespo, J.L., M.G. Guereo, and F.J. Florencio. 1999. Mutational analysis of Asp51 of *Anabaena azollae* glutamine synthetase. D51E mutation confers resistance to the active site inhibitors L-methionine-DL-sulfoximine and phosphinothricin. European Journal of Biochemistry 266:1202-1209.

Duke, S.O. 1998. *In* Herbicides: Chemistry, Degradation and Mode of Action; Kearney, P.C. & Kaufman, D.D. (Eds.), Marcel Decker Inc., New York, 1988, 3: 1-70.

Franz, J.E., M.K. Mao, and J.A. Sikorski. 1997. *In* Glyphosate A Unique Global Herbicide. ACS Monograph 189. 1997 American Chemical Society, Washington, D.C.

Heap, I. 2003. www.weedscience.com

Heck, G.R., S.Y. Chen, T. Chiu, P. Feng, J. Huang, C.S. Hubmeier, Y. Qi, and R.D. Sammons. 2002. Ongoing investigations in to glyphosate resistant horseweed. Proc. N. Cent. Weed Sci. Soc. 57:Addendum.

Herbicide Resistance Action Committee. 2002. Classification of herbicides according to mode of action. http://www.plantprotection.org/HRAC/.

Holt, J.S. and H.M. Le Baron. 1990. Significance and distribution of herbicide resistance. Weed Technology 4:141-149.

Jander, G., S.R. Baerson, J.A. Hudak, K.A. Gonzalez, K.J. Gruys, and R.L. Last. 2003. Ethylmethanesulfonate saturation mutagenesis in Arabidopsis to determine frequency of herbicide resistance. Plant Physiology 131:139-146.

Le Baron, H. M. 1991. Herbicide resistant weeds continue to spread. Resistant Pest Management Newsletter 3:36-37.

Lorraine-Colwill, D. F., S.B. Powles, T.R. Hawkes, P.H. Hollinshead, S.A.J.. Warner. and C. Preston. 2003. Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. Pest. Biochem. Physiol. 74:62-72.

Malik, J., G. Barry, and G. Kishore. 1989. Mini review: The herbicide glyphosate. BioFactors Vol. 2 no.1:17-25.

Matthews, J.M. 1994. Management of herbicide resistant populations. *In* Herbicide Resistance in Plants, Biology and Biochemistry; S.B. Powles and J.A.M. Holtum (eds.). Lewis, Ann Arbor. pp. 317-335.

Maxwell, B. D. and A.M. Mortimer. 1994. Selection for herbicide resistance. *In* Herbicide Resistance in Plants, Biology and Biochemistry; S.B. Powles and J.A.M. Holtum (eds.). Lewis, Ann Arbor. pp. 1-26.

Ng, C.H., R. Wickneswari, S. Salmijah, Y.T. Teng and B.S. Ismail. 2003. Gene polymorphisms in glyphosate-resistant and –susceptible biotypes of *Eleusine indica* from Malaysia. Weed Research 43:108-115.

OECD. 1999. 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. http://www.oecd.org/ehs/

Pratley, J., P. Baines, P. Eberbach, M. Incerti, and J. Broster. 1996. Glyphosate resistance in annual ryegrass. Proc. Eleventh Ann. Conf. Grassld. Soc. NSW, pp. 122.

Powles, S.B., and J.A.M. Holtum. 1994. *In* Herbicide Resistance in Plants, Biology and Biochemistry. Lewis, Ann Arbor. pp. 353.

Roush, M.L., S.R. Radosevich, and M.B. Maxwell. 1990. Future outlook for herbicide-resistance research. Weed Technology 4:208-214.

Schonbrunn, E., S. Eschenburg, W.A. Shuttleworth, J.V. Schloss, N. Amrhein, J.N.S. Evans, and W. Kabsch. 2001. Interaction of the herbicide glyphosate with its target enzyme EPSP synthase in atomic detail. Proc. Natl. Acad. Sci. U.S.A. 98:1376-1380.

Shaner, D.L. 1995. Herbicide Resistance: Where are we? How did we get here? Where are we going? Weed Technology 9:850-856.

Steinrücken, H.C., and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochem. Biophys. Res. Commun. 94 (4):1207-1212.

Steinrücken, H.C. and N. Amrhein. 1984. 5-enolpyrvylshikimate-3-phophate synthase of Klebsiella pneumonia. Eur. J. Biochem. 143:351-357.

Stoltenberg, D. E. 2002. Weed Management and Agronomic risks associated with glyphosate-resistant corn and soybean cropping systems. Proceedings of the 2002 Wisconsin Fertilizer, Aglime and Pest Management Conf. 41:200-208.

Trannel, P. and T.R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Science 50:700-712.

VanGessel, M.J. 2001. Glyphosate resistant horseweed from Delaware. Weed Science 49:703-705.

Widholm, J.M., A.R. Chinnala, J.H. Ryu, H.S. Song, T. Eggett, and J.E. Brotherton. 2001. Glyphosate selection of gene amplification in suspension cultures of 3 plant species. Physiol. Plant. 112:540-545.

Wilson, R.G. and P. Stahlman. 2003. Maintain the rate. pp. 12-13, Nebraska Farmer, June edition.

APPENDIX 2

COMMUNICATION FROM USDA-ARS ON U.S. WEEDY RELATIVES OF SUGAR BEET

United States Department of Agriculture

Research, Education and Economics
Agricultural Research Service

September 25, 2003

Dr. J. R. Stander
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Dear Dr. Stander,

I have been giving a lot of consideration to your request to comment on the occurrence and potential problems with "weed beet" in the United States, especially in relation to the potential for hybridization between herbicide-resistant sugar beet and any weed beet. I have contacted both public and private weed scientists and researchers who work with sugar beet in all of the sugar beet growing regions of the United States to assure that I was properly informed before commenting. Let me give you a little background for those who may not be as familiar with sugar beet as yourself.

Normally sugar beet (*Beta vulgaris* subspecies *vulgaris*) is a biennial crop that remains vegetative and forms a fleshy taproot as a storage organ (the agronomic crop) in the first year. The plant must undergo a period of cold temperature vernalization before it can enter its reproductive phase, and, in the second year, the sugar beet uses the stored sugar to produce a flower stalk and set seed. If the spring weather is especially cool, some of the sugar beet plants may vernalize in the seedling stage and bolt - i.e., put up a flower stalk in the first year, and sometimes these will set a little seed. These seed have the potential to become weeds in following crops. Additionally, some of the wild relatives of sugar beet, especially those in the subspecies *maritima* that are sexually compatible (i.e. can form fertile hybrids) with sugar beet, have an annual reproductive cycle. These would have the potential to become weeds, and, indeed, are a serious weed problem in parts of Europe where they are native. None of those are, however, native to the United States, and the only area in which they might be present is California. I do not know of any other plant species (outside of *Beta vulgaris* subspecies and *Beta macrocarpa*) in the United States that are sexually compatible with sugar beet.

There have been reports in the literature, that sugar beet has bolted and produced plants from seed the following year. In our rotations, however, sugar beet is generally planted only every third year and is easily controlled by most broadleaf herbicides, indeed, if the weather conditions are right, even some of those herbicides that are registered for use on sugar beet can cause considerable damage. Our winter weather in most sugar beet growing areas will not allow the root to survive, and any plants produced by seed from bolters do not persist long in the environment. Sugar beet has been cultivated in the Northern High Plains and many other parts of the United States for well

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over 100 years, and, in that time, no weed beet problem has ever occurred. And today, we have much better varieties, with fewer bolters, than was the case even thirty-five years ago.

The story in California is a little bit different due to the climate and historical introduction of cultivated beet and wild beet by the Spanish and Portugese. I have talked with Dr. R. T. Lewellen, an ARS geneticist who has worked with sugar beet at the USDA-ARS Salinas Research Station for many years. He is familiar with the situation there, and what he reports agrees with what I have seen reported in the literature and heard from others.

There are a wild beet populations, including the so-called Milpitas wild beet, in the San Francisco Bay area and south along the coast of California. It was speculated that these wild beets are most likely a mixture of escaped and annualized cultivated beet (Red Garden Beet and Swiss Chard), introduced by the early Spanish settlers, with escaped sugar beet from the early sugar beet culture in this area (which began in the last half of the 1800s). The work done by Drs. Detlef Bartsch and Norman Ellstrand in the mid 1990s looking at gene frequencies (isozyme loci) has confirmed that these are mixed populations of *Beta vulgaris* ssp. *vulgaris* and *Beta vulgaris* ssp. *maritima* (wild sea beet). They are, however, in areas in which sugar beet is no longer grown in California.

There is also a population of wild weed beets in the Imperial Valley of California. These were noted by Carsner in 1928 and again by John McFarlane, the USDA-ARS geneticist and sugar beet breeder, in the mid 70s. Because beets were not grown in this area until the late 1930s, they can't have been annualized sugar beets, which escaped from cultivated populations. As a matter of fact, based on the their morphology, McFarlane identified these wild beets as another species of *Beta*, *B. macrocarpa*. He thought that they might have been introduced in the early settlement of Imperial Valley from the Canary Islands by Portugese immigrants. Bartsch and Ellstrand confirmed that these were indeed *B. macrocarpa*, and were almost identical in isozyme allele frequencies to *B. macrocarpa* found in Spain, which supports Dr. McFarlane's speculations.

These populations are a weed problem only in sugar beet grown in the Imperial valley, because herbicides that are safe to use on sugar beet do not harm this close relative of sugar beet. Dr. Lewellen has done some research on this species and it is his opinion that it does not outcross readily to sugar beet. There are a number of factors supporting this conclusion. First, these plants usually bolt and flower too early to hybridize with sugar beet - their seed has matured before sugar beet bolts and flowers in May to June. *B. macrocarpa* is somewhat compatible in crosses with sugar beet but in F_1 hybrids made by Dr. Lewellen, the plants were mostly pollen sterile, and the F_2 plants had very disturbed genetic ratios and growth habit. He feels that they would not survive well in nature. Additionally, these populations of *B. macrocarpa* are self-fertile. Even in the greenhouse, crosses of *B. macrocarpa* and sugar beet could only be made with sugar beet as the female, either using self-sterile or male sterile sugar beet plants. In nature, this would not happen

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because the flowering period of bolted sugar beet comes much later in spring than that the flowering of *B. macrocarpa*, and these flowering periods do not normally overlap. McFarlane found one population in the Imperial Valley which showed signs of having outcrossed to sugar beet. Again this was confirmed by Bartsch and Ellstrand, who could only find one population of *B. macrocarpa* that showed evidence of hybridization with sugar beet, and at a rate of only 2% over a almost seventy year time span.

As Dr. Lewellen notes, after more than 100 years of sugar beet production and breeding programs in the Salinas Valley of California, where winter planted sugar beet has often bolted and produced hard seed, no wild beet problem is known. Nor has there been obvious outcrosses of wild beets into their seed isolation plots used to make line increases and experimental hybrids.

In seed production areas of Europe (England, France, and Italy), the wild sea beet (*Beta vulgaris* ssp. *maritima*) is naturally occurring, and readily forms fertile hybrids with sugar beet (and other cultivated beet types). There have been a number of recent studies on gene flow between these seed production plots and wild beets in Europe, and this is where the largest problem is seen. The entire United States production of sugar beet seed is in Oregon, and wild or weed beets are not known to occur in Oregon.

The data indicate that there is very little risk of a transgenic (herbicide-resistant) sugar beet hybridizing with a weed beet population, and, if so, only in the Imperial Valley of California. And in the remote possibility that this would happen, it is unlikely that these wild beets would be a potentially larger weed problem than they currently are, in fact, this might be the one way to manage these weed beets effectively. There are no other persistent wild beet populations known anywhere in the United States, outside of the California coastal area, where sugar beet is no longer grown. Sugar beet could be controlled with many classes of herbicides and even a herbicide-resistant weed beet population, if it could persist under our climatic conditions, could be easily controlled before it became a serious weed problem.

Sincerely yours,

Lee Panella /s/
Chair, Sugarbeet Crop Germplasm Committee

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May 28, 2004

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Re: Review for Completeness and Acceptability: Roundup Ready® Sugar Beet Event H7-1: Petition for Determination of Nonregulated Status.

Monsanto Company and KWS SAAT AG, the submitters, have received a letter of review for completeness and acceptability regarding the Roundup Ready sugar beet event H7-1 petition #03-323-01p from USDA-APHIS on May 14, 2004. In response to the letter of review for completeness and acceptability, this document is being provided to USDA-APHIS to address the questions.

Question 1. p.108: Table VII-12. Please verify the completeness of this table. Two items may be missing from the list of resistant weeds in Minnesota.

Response: The submitters agree that Table VII-12 is missing two documented herbicide-resistant weeds identified in the state of Minnesota. Refer to the updated Table VII-12 below for a complete listing of the documented herbicide-resistant weeds in key sugar beet producing states in the U.S.

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6/2/04
Kay

Table VII-12. Herbicide-resistant weeds identified in key sugar beet producing states in the U.S.[1]

Idaho

Species	Common Name	Year[2]	Herbicide Class
1. *Lactuca serriola*	Prickly Lettuce	1987	ALS inhibitors
2. *Kochia scoparia*	Kochia	1989	ALS inhibitors
3. *Salsola iberica*	Russian Thistle	1990	ALS inhibitors
4. *Avena fatua*	Wild Oat	1992	ACCase inhibitors
5. *Lolium multiflorum*	Italian Ryegrass	1992	ACCase inhibitors
6. *Avena fatua*	Wild Oat	1993	Thiocarbamates
7. *Avena fatua*	Wild Oat	1993	Pyrazoliums
8. *Anthemis cotula*	Mayweed Chamomile	1997	ALS inhibitors
9. *Kochia scoparia*	Kochia	1997	Synthetic Auxins

Michigan

Species	Common Name	Year[2]	Herbicide Class
1. *Chenopodium* album	Lambsquarters	1975	Photosystem II inhibitors
2. *Ambrosia artemisiifolia*	Common Ragweed	1990	Photosystem II inhibitors
3. *Senecio vulgaris*	Common Groundsel	1990	Photosystem II inhibitors
4. *Portulaca oleracea*	Common Purslane	1991	Photosystem II inhibitors
5. *Portulaca olercea*	Common Purslane	1991	Ureas and amides
6. *Daucus carota*	Wild Carrot	1993	Synthetic Auxins
7. *Ambrosia artemisiifolia*	Common Ragweed	1998	ALS inhibitors
8. *Amaranthus tuberculata*	Tall Waterhemp	2000	ALS inhibitors
9. *Amaranthus powellii*	Powell Amaranth	2001	Photosystem II inhibitors
10. *Amaranthus powellii*	Powell Amaranth	2001	Ureas and amides
11. *Amaranthus retroflexus*	Redroot Pigweed	2001	Photosystem II inhibitors
12. *Amaranthus retroflexus*	Redroot Pigweed	2001	Ureas and amides
13. *Chenopodium album*	Lambsquarters	2001	ALS inhibitors
14. *Polygonum persicaria*	Ladysthumb	2001	Photosystem II inhibitors
15. *Amaranthus hybridus*	Smooth Pigweed	2002	ALS inhibitors
16. *Conyza canadensis*	Horseweed	2002	ALS inhibitors
17. *Conyza canadensis*	Horseweed	2002	Photosystem II inhibitors
18. *Conyza canadensis*	Horseweed	2002	Ureas and amides

Minnesota

Species	Common Name	Year[2]	Herbicide Class
1. *Chenopodium album*	Lambsquarters	1982	Triazines
2. *Abutilon theophrasti*	Velvetleaf	1991	Triazines
3. *Amaranthus retroflexus*	Redroot Pigweed	1991	Triazines
4. *Avena fatua*	Wild Oat	1991	ACCase inhibitors
5. *Kochia scoparia*	Kochia	1994	ALS inhibitors
6. *Xanthium strumarium*	Common cocklebur	1994	ALS inhibitors
7. *Setaria faberi*	Giant Foxtail	1996	ALS inhibitors
8. *Setaria viridis*	Robust White Foxtail (var. *robusta-alba Schreiber*)	1996	ALS inhibitors
9. *Setaria lutescens*	Yellow Foxtail	1997	ALS inhibitors
10. *Ambrosia artemisiifolia*	Common Ragweed	1998	ALS inhibitors
11. *Setaria viridis*	Robust White Foxtail (var. *robusta-alba Schreiber*)	1999	ACCase inhibitors
12. *Setaria viridis*	Robust Purple Foxtail (var. *robusta-purpurea*)	1999	ACCase inhibitors

Table VII-12 (continued). Herbicide-resistant weeds identified in key sugar beet producing states in the U.S.[1]

North Dakota

Species	Common Name	Year[2]	Herbicide Class
1. *Kochia scoparia*	Kochia	1987	ALS inhibitors
2. *Setaria viridis*	Green Foxtail	1989	Dinitroanilines and others
3. *Avena fatua*	Wild Oat	1991	ACCase inhibitors
4. *Kochia scoparia*	Kochia	1995	Synthetic Auxins
5. *Avena fatua*	Wild Oat	1996	ALS inhibitors
6. *Kochia scoparia*	Kochia	1998	Triazines
7. *Amaranthus retroflexus*	Redroot Pigweed	1999	ALS inhibitors
8. *Sinapis arvensis*	Wild Mustard	1999	ALS inhibitors
9. *Solanum ptycanthum*	Eastern Blk.Nightshade	1999	ALS inhibitors

Legend:

[1] Source: Heap, I. The International Survey of Herbicide-resistant Weeds. Online. Internet. Available at www.weedscience.com.

[2] Year resistance was first reported.

Question 2. p. 116: You make note that significant sugar beet seed production takes place in the Willamette Valley of Oregon. Please briefly discuss other substantive U.S. seed production areas outside of Oregon.

Response: Presently, sugar beet seed multiplication in the U.S. is produced predominately in the Willamette Valley of Oregon. There is also some production in other regions of Oregon, including the smaller valleys South of the Willamette Valley. There is also limited seed production in the Madras area of Central Oregon, with production in these areas typically occurring on a trial basis and considered supplemental to those in the Willamette Valley. Outside of these areas in Oregon, any sugar beet seed production is relatively small, consisting primarily of breeder seed and experimental hybrid seed productions associated with research stations of private sugar beet breeding companies or USDA-ARS stations.

Sugar beet seed multiplication has also historically been produced in the Salt Lake Basin of Utah. Currently, there is no seed production in the Salt Lake Basin in Utah, but there exists the potential for seed production to occur in this area again in the future.

Question 3. p. 130 and 131: The Weed Science Society of America has recently made additions to their website in the list of glyphosate resistant weeds (http://www.weedsceince.org/summary/MOASummary.asp). You should address these additions and the extent to whether these would or would not impact growing of Roundup Ready® Sugar Beet in the U.S.

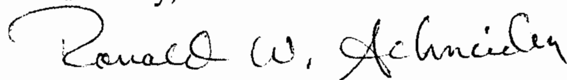
Response: To date, on a global basis, biotypes of only four weed species resistant to glyphosate have been confirmed through field and laboratory testing by Monsanto. Recently, populations of two weed species in South Africa, hairy fleabane (*Conyza bonariensis*) and buckhorn plantain (*Plantago lanceolata*), have been reported as resistant to glyphosate (Heap, 2003). Monsanto is investigating these populations and has not confirmed resistance based on field and laboratory testing at this time. Various herbicides are available for control of these species, but they do not commonly occur in U.S. sugar beet production.

References:

Heap, I. The International Survey of Herbicide Resistant Weeds. Online. Internet. May 17, 2004. www.weedscience.com.

Should you have additional questions with regard to the information provided by the submitters in this response document to the letter of review for completeness and acceptability regarding a request for a determination of nonregulated status for Roundup Ready sugar beet event H7-1, please contact either Dr. Russell P. Schneider, Director, Regulatory Affairs, at 202-383-2866, or me at 314-694-3263.

Sincerely,



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