AgrEvo

97-336-01p

January 23, 1998

Dr. Ray Dobert
Animal and Plant Health Inspection Svcs.
Biotechnology, Biologics, and Environmental Protection
Biotechnology Permits
4700 River Rd., Unit 147
Riverdale, MD 20737-1237

Re: Petition 97-336-01p

Dear Dr. Dobert:

Please find enclosed two (2) copies each of replacement pages 16, 20, 24, 27 and 32 for AgrEvo® petition **97-336-01p**. These pages have been amended per our telephone discussion on January 23, 1998.

If you have any questions regarding these changes, please do not hesitate to contact me at 302-892-3034.

Your attention to our efforts to gain USDA Nonregulated Status are much appreciated.

Sincerely,

Vickie Forster

Vickie Forster
Registration Specialist
Regulatory Affairs - Biotechnology

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AgrEvo USA Company
Little Falls Centre One, 2711 Centerville Road, Wilmington, DE 19808, Telephone: (302) 892-3000, Fax: (302) 892-3013

1/27/98

AgrEvo

97-336-01p

January 19, 1998

Dr. Ray Dobert
Animal and Plant Health Inspection Svcs.
Biotechnology, Biologics, and Environmental Protection
Biotechnology Permits
4700 River Rd., Unit 147
Riverdale, MD 20737-1237

Re: Petition 97-336-01p

Dear Dr. Dobert:

Please find enclosed two (2) original copies of replacement pages 6-69, inclusive for AgrEvo® petition **97-336-01p**. Please replace the original pages with these pages and return to AgrEvo® the replaced pages.

Please feel free to contact me at 302-892-3034 with any questions.

Your attention to our petition is very much appreciated.

Sincerely,

Vickie Forster \ sij

Vickie Forster
Registration Specialist
Regulatory Affairs - Biotechnology

AgrEvo USA Company
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1/29/98

AgrEvo

97-336-01p

December 1, 1997

Mr. Michael A. Lidsky
Deputy Director
USDA, APHIS, PPQ, BSS, CTA
4700 River Rd. Unit 146
Riverdale, MD 20737-1237

Re: Petition for Determination of Nonregulated Status: Gulfosinate-Ammonium Tolerant Sugar beet Transformation Event T120-7

Dear Mr. Lidsky:

AgrEvo USA Company herein submits a Petition for Determination of Nonregulated Status for Gulfosinate-Ammonium Tolerant Sugar beet Transformation Event T120-7.

This petition requests a determination from USDA/APHIS that glufosinate-ammonium tolerant sugar beet transformation event T120-7, and any progeny derived from breeding other sugar beet lines with event T120-7, no longer be considered regulated articles under 7 CFR Part 340. This petition contains a full statement explaining the factual grounds why glufosinate-ammonium tolerant sugar beet transformation event T120-7 should not be regulated under 7 CFR 340.6. This petition does not contain any trade secrets or confidential business information (CBI) and is so marked.

Please find enclosed the following documents:

Two copies of the Petition for Determination of Nonregulated Status: Glufosinate-Ammonium Tolerant Sugar beet Transformation Event T120-7, including Appendicies I-V.

AgrEvo USA Company
Little Falls Centre One, 2711 Centerville Road, Wilmington, DE 19808, Telephone: (302) 892-3000, Fax: (302) 892-3013

Do not hesitate to contact me at 302-892-3034, phone; or, 302-892-3099, fax, with any questions regarding this petition.

Your consideration of this petition is greatly appreciated.

Sincerely,

Vickie Forster

Vickie Forster
Registration Specialist, Biotechnology

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97-336-01p

Petition for Determination of Nonregulated Status:

Glufosinate Tolerant Sugar Beet, Transformation Event T120-7

Submitted by:

Vickie Forster December 1, 1997

Vickie Forster
Registration Specialist, Regulatory Affairs-Biotechnology

AgrEvo USA Company
Little Falls Centre One
Centerville Road
Wilmington, DE 19808
Telephone: 302-892-3034
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Contributors:

Dick Marrese, Kevin Staska, Klaus Trinks, J.R. Stander

December 1, 1997

12/2/97

SUMMARY

AgrEvo USA Company, Wilmington, Delaware, herein submits a Petition for Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS), of the United States Department of Agriculture (USDA), for Glufosinate Tolerant Sugar Beet Transformation Event **T120-7**. AgrEvo requests a determination from APHIS that transformation event T120-7, any progeny derived from event T120-7 that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340. Event T120-7 is considered a regulated article because it contains DNA sequences from the plant pest, cauliflower mosaic virus (CaMV); the plant pest, *Agrobacterium tumefaciens*; and, was transformed using the plant pest *A. tumefaciens*.

Glufosinate-ammonium is in the glutamine synthetase inhibitor class of herbicides. It is a non-systemic, non-selective herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. Glufosinate-ammonium controls weeds through the inhibition of glutamine synthetase, which leads to the accumulation of phytotoxic levels of ammonia in the plant. Glutamine synthetase is the only enzyme in plants that can detoxify ammonia released by photorespiration, nitrate reduction, and amino acid degradation.

Transformation event T120-7 is sugar beet, *Beta vulgaris*, material containing a stably integrated gene which encodes the enzyme phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme catalyzes the conversion of L-phosphinothricin, the active ingredient in glufosinate-ammonium, to an inactive form, thereby conferring tolerance to the herbicide. The *pat* gene in event T120-7 is a synthetic version of the native gene isolated from *Streptomyces viridochromogenes*, strain Tü 494. The nucleotide sequence has been modified to provide codons preferred by plants without changing the amino acid sequence of the enzyme. The gene was introduced into sugar beet calli using disarmed *A. tumefaciens*. Southern blot and analyses show that event T120-7 contains a single, stably integrated copy of the *pat* gene. Southern blot and plymerase chair reaction (PCR) analyses confirm that the incorporation has been limited to DNA sequences contained within the T-DNA borders.

Genetically engineered glufosinate tolerant sugar beet will provide a new weed management tool to sugar beet growers. Glufosinate-ammonium is currently registered in the United States as a herbicide for both non-crop and crop uses. It is registered as FINALE® for non-crop uses; as RELY® for use on trees, nuts and vines; as REMOVE™ for seed propagation use, currently on corn and soybean; and as LIBERTY™ for crop use, currently on corn and soybean. Glufosinate-ammonium is biodegradable, has no residual activity, and has very low toxicity for humans and wild fauna. Glufosinate tolerant sugar beet may positively impact current agronomic practices in sugar beet by, **1)** offering a broad spectrum, post-emergence weed control system; **2)** providing the opportunity to continue to move away from pre-emergent and residually active compounds; **3)** providing a new herbicidal mode of action that allows for improved weed resistance management in sugar beet acreage; **4)** offering the use of an environmentally sound and naturally occurring herbicide; **5)** encouraging herbicide use on an as needed basis; **6)** decreasing cultivation

ring herbicide; 5) encouraging herbicide use on an as needed basis; 6) decreasing cultivation needs; and 7) allowing the application of less total pounds of active ingredient than used presently in sugar beet.

Transformation Event T120-7 has been field tested in the United States during the 1994 - 1997 growing seasons. In 1994 three (3) field trials were conducted under USDA permit 94-054-06r; in 1995 six (6) field trials were conducted under USDA permit 94-347-01r; and, in 1996 nineteen (19) field trials were conducted under USDA permit 96-052-02r. In 1997 forty-four (44) field trials were conducted under USDA permit 97-029-01r. All trials were conducted during the growing season in primary sugar beet growing States. Transformation event T120-7 has also been field tested extensively in Western and Eastern Europe, the Former Soviet Union, and Canada.

Data collected from field trials, laboratory analyses, and literature references presented herein demonstrate that glufosinate-tolerant sugar beet event T120-7:

- exhibits no plant pathogenic properties,
- is no more likely to become a weed than non-modified sugar beet,
- is unlikely to increase the weediness potential of any other cultivated plant or native wild species,
- does not cause damage to processed agricultural commodities,
- is unlikely to harm other organisms that are beneficial to agriculture.

Transformation event T120-7 has been selected for commercial development. It has been crossed with available traditionally derived sugar beet lines. The primary transformation event T120-7 and its progeny are collectively referred to as glufosinate-tolerant sugar beet T120-7 in this petition.

Consultation with the Food and Drug Administration (FDA) is underway regarding the food and feed safety of event T120-7. A submission for registration of the herbicide, glufosinate-ammonium, for use on sugar beet has been made to the Environmental Protection Agency (EPA).

CERTIFICATION

The undersigned certifies, 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 relevant data and information known to the petitioner which are unfavorable to the petition.

Vickie Forster

Vickie Forster
Registration Specialist, Regulatory Affairs-Biotechnology

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ACRONYMS AND SCIENTIFIC TERMS

APH (3') II: aminoglycoside (3') phosphotransferase type II

***aph (3') II*:** aminoglycoside (3') phosphotransferase type II gene

APHIS: Animal and Plant Health Inspection Service (APHIS)

CaMV: cauliflower mosaic virus

CMS: cytoplasmic male sterility

DNA: deoxyribonucleic acid

ELISA: enzyme linked immunosorbant assay

EPA: United States Environmental Protection Agency

FDA: United States Food and Drug Administration

NaOH: sodium hydroxide

PAT: phosphinothricin acetyltransferase

***pat*:** phosphinothricin acetyltransferase gene (origin *Streptomyces viridochromogenes*)

PCR: polymerase chain reaction

SDS: sodium dodecylsulfate

T-DNA: DNA between the left and right borders of a Ti plasmid; the DNA transferred to the plant genome

***tet*:** gene conferring resistance to the antibiotic tetracycline

USDA: United States Department of Agriculture

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I. Rationale for Development of Glufosinate Tolerant Sugar Beet, Transformation Event T120-7

Sugar beet, *Beta vulgaris*, is grown for sugar production primarily in the northern climes of Europe and North America. Sugar beet for sugar production is also grown in Asia and the Middle East. Sugar production from sugar beet grown in the United States accounts for **3.1%** of the total production of sugar worldwide. European (including the European Union, Former Soviet Union 12 and Eastern Europe) sugar beet production accounts for **22.8%** of the total production of sugar worldwide. Sugar cane grown in tropical regions, accounts for approximately **74%** of the total worldwide sugar production. All refined sugar and molasses produced from sugar beet in the USA is consumed within the population. No sugar is exported. Sugar produced in Europe and tropical regions is exported throughout the rest of the world. For more information regarding sugar production and consumption throughout the world, consult USDA Agricultural Statistics (1997).

Several herbicides are currently available to the grower for weed management in sugar beet. Weed management is critical to achieve maximum yield for the sugar beet grower. The grower is typically interested in applying a herbicide for weed control that has a broad weed spectrum, does not injure the crop, is cost effective, and has positive environmental attributes. Several classes of herbicides have effective broad spectrum weed control if used either singly or in combination, however, they may injure or kill some crops depending on environmental conditions around the time of application.

Glufosinate-ammonium, the active ingredient in Liberty™ Herbicide, and in the Liberty Link™ seed system, is a broad spectrum, non-systemic herbicide. It has very favorable environmental and ecological impact characteristics such as low residual activity, little soil leaching, and low toxicity to non-target organisms. Tolerance to glufosinate-ammonium has been achieved through the genetic engineering of over twenty (20) commercially important crop species, including sugar beet. Liberty Link™ sugar beet will provide a selective use for glufosinate-ammonium and a valuable new weed management tool for sugar beet growers.

Statement of Grounds for Nonregulated Status

II. The Sugar Beet Family

A. History and Uses of Sugar Beet

The sugar beet root was a common element of the Egyptian diet during the building of the pyramids. Its use as a source of sugar, however, was not discovered until the middle of the 18th Century in Europe (Smith, 1980). In 1747, the German Chemist, Marggraf discovered that the sweet tasting crystals obtained from white beet roots were identical to those obtained from sugar cane. Marggraf's extraction of macerated roots produced 1.6% crystallized sugar. While he reported these results, Marggraf focused his attention on other research projects because of the low recovery factor. A student of Marggraf's, Franz Carl Achard, continued the investigation of extracting white beet roots which led to the concept of sugar being produced from the white skinned Silesian *Beta vulgaris* root. Achard constructed a small processing plant in 1801-02 which later became a school for sugar production. Achard is today considered the father of the sugar beet industry (Smith, 1987; Cooke and Scott, 1993; and Coons, Owen and Stewart, 1955).

The first sugar beet processing plant in the United States was built in Northampton, Massachusetts in 1838. Many processing plants followed, but most were short lived. In 1870, a processing plant was built in Alvarado, California, which became the forerunner of the U.S. sugar beet processing industry. Several additional plants were constructed across the USA in the sugar beet growing regions during the late 19th century. As plants became more efficient, fewer were needed, and by 1994-95, only 34 factories processing sugar beets remained active (Panella 1996). Today, 26% of the world sugar consumed and 47% of the U.S. sugar consumed is produced from sugar beets (USDA Agricultural Statistics, 1997).

In the United States, there are five (5) major sugar beet growing regions (regions are listed in order of sugar beet acreage): **1)** the Red River Valley of Minnesota and North Dakota, **2)** Southern Idaho, **3)** the "Intermountain" region, consisting of parts of Montana, Wyoming, Nebraska and Colorado, **4)** the Imperial and Central Valleys of California, and **5)** the "thumb" of Michigan. In the semi arid areas of the western region, including Montana, Wyoming, Nebraska, Colorado, Texas, Idaho, Oregon, Washington, and California, inadequate spring and summer rains necessitate irrigation. In California, irrigated sugar beets are grown under a wide range of soil and climatic conditions and may be grown as a summer or winter crop (Welch, 1997).

In 1996, in the USA, sugar beets were grown (harvested) on 1,322,900 acres, with more than 99% grown primarily in twelve states. Minnesota and North Dakota grew 663,300 acres (50%). The Imperial Valley of California and Southern Idaho together grew 266,000 acres (20%), Michigan grew 130,000 acres (10%), and the Intermountain states of Colorado, Nebraska, Montana, Wyoming grew 16.4%. Thus, in 1996, these five regions accounted for 96.4% of the United States sugar beet production (USDA Agricultural Statistics, 1997). Since 1973, there has

been no price protection to the sugar beet industry in the U.S. Many farmers have switched to alternate crops. Only in the Red River Valley of North Dakota and Minnesota, and in the state of Washington, has there been any expansion of crop acreage in the sugar beet industry. Sugar beets in these states can be grown more economically than other areas (Cooke and Scott, 1993).

Each sugar beet root consists of 75.9% water, 2.6% non-sugars, 16.0% sugar and 5.5% pulp. In the sugar fraction 83.1% is recovered as crystalline sucrose, 12.5% is recovered as molasses. The remaining 4.4% are impurities (Bichsel, 1987). Many non-sucrose components of sugar beet roots affect the crystallization of sugar during processing which accounts for a loss of sucrose. These compounds include an organic nitrogen base, betaine, and potassium and sodium salts (Smith, 1987b).

The by-products from the processing of sugar beets include sugar beet pulp and molasses. Sugar beet pulp is dried and pelleted for use as a livestock feed. Molasses is also used as livestock feed to make feedstuffs such as hay more palatable. Sugar beet tops are used in a limited amount as livestock feed (cattle, sheep) when they are left in fields for grazing. Sugar beet pulp has an affect on dairy cow milk fat content, where it has been shown that it significantly increases the fat content compared with a barley-based diet (Mayne and Gordon, 1984). However, dairy cow milk yields for a barley-based versus a sugar beet pulp-based diet were not significantly different.

B. Taxonomy and Habit of Sugar Beets

Sugar beets are a member of the family *Chenopodiaceae* (Goosefoot Family). This family includes three other groups cultivated by man: the leaf beet (Swiss chard), the red table beet and mangolds (fodder beet), from which the sugar beet was derived (Cooke and Scott, 1993; Coons, Owen and Stewart, 1955). Members of this family are dicotyledonous and usually herbaceous in nature. They are also halophytes and many of the family's weed species are found along sea coasts and near brackish marshes. Several members of this family may also be found invading crops. These include Lambsquarters (*Chenopodium album*), Mexican fireweed (*Kochia scoparia*) and Russian thistle (*Salsola kali*) (Fernald, 1950; University of Illinois Bulletin 772, 1981). Agriculturally important plants of the genus *Beta* belong to the species *vulgaris*. The genus *Beta*, including the wild relatives, is divided into four sections as shown in Table 1. (Smith 1987a; Panella 1996):

TABLE 1: Species of the Genus *Beta*

SECTION	SPECIES	CHROMOSOME NUMBERS
Beta	*B. vulgaris* L.	
	subsp. *vulgaris* L.	2n = 2x = 18
	subsp. *maritima* L.	2n = 2x = 18
	subsp. *adanensis* (Pamuk.)	2n = 2x = 18
	B. macrocarpa (Guss.)	2n = 2x = 18
	B. patula (Ait.)	2n = 2x = 18
Corollinae	*B. lomatogona* (F.et Mey)	2n = 2x = 18, 4x = 36
	B. trigyna (W. et Kit)	2n = 4x = 36, 5x =45, 6x = 54
	B. corolliflora	2n = 2x = 18
	B. macrochiza (Stev.)	2n = 4x = 36
	B. intermedia (Bunge)	2n = 2x = 18
Procumbentes	*B. patellaris* (Moq.)	2n = 4x = 36
	B. procumbens (Chr. Sin.)	2n = 2x = 18
	B. webbiana (Moq.)	2n = 2x = 18
Nanae	*B. nana* (Boiss. & Hldr.)	2n = 2x = 18

Beta is considered an Old World genus basically confined to the Mediterranean Basin and Middle East. The genus has been organized into four sections: *Beta* (formerly *Vulgares*), *Corollinae*, *Procumbentes* (formerly *Patellares*), and *Nanae*. The taxonomy of the *Beta* section has recently been revised. This proposal was based upon morphometric analysis of variation, allozyme differentiation, and evaluation of available herbarium specimens. The section consists of three species: *B. vulgaris*, a large and variable species containing both cultivated and wild materials; *B. macrocarpa;* and *B. patula. Beta vulgaris* is subdivided into three subspecies: *subsp. vulgaris*, containing all cultivated materials; *subsp. maritima,* a large and variable group of plant types; and subsp. *adanensis*. Further subdivision was not considered of value. The so-called weed beets are classified as *B.vulgaris ssp.maritima.*

The sugarbeet, as originally developed, was a diploid with 18 chromosomes (2x). After discovery of the chromosome doubling properties of colchicine considerable experimenting was done with polyploid sugarbeets. Commercial exploitation of polyploidy in sugarbeets began in Europe in the 1940s with the development of anisoploid varieties. Such varieties were actually mixtures including diploid, triploid and tetraploid individuals, and were produced by interpollination of diploid and tetraploid seed-parents. The use of cytoplasmic sterility in conjunction with polyploidy allowed the production of triploid varieties. Currently there are diploid, triploid, and anisoploid varieties available. Sugarbeet plants with higher ploidy levels have been observed and

have been produced experimentally, notably auto-hexaploids and auto-octoploids, but have had limited usefulness.

The agriculturally important sugar beet, *B. vulgaris*, is a herbaceous dicot which usually completes its life cycle in two years. This biennial characteristic is somewhat variable and under certain conditions sugar beet can act as an annual (Smith, 1987a). Wild populations of *B. vulgaris* consist of annual, biennial and perennial species, but only the biennial type has been developed for sugar beet production (Hecker and Helmerick, 1985). Under good conditions, with scheduled planting dates, sugar beets produce a fleshy, bulbous taproot during the first growing season which is the sugar producing crop. Following an overwintering period of cold temperature (4-7°C) the sugar beet plant sends up a flowering stalk the second growing season which is the basis of the seed crop (Smith, 1987a).

Sugar beets are sown to achieve a plant population of 75,000 plants per hectare (approximately 30,000 plants per acre) (Cooke and Scott, 1993). Initial growth of the sugar beet seedling shows a greater early development of leaf tissue. Six weeks following emergence, the plant has 8-10 leaves and still has a very small root. From this stage on, both leaf and root tissue develop simultaneously with the root eventually becoming a greater proportion of the plant's dry weight (Cooke and Scott, 1993). The sugar beet root develops in a series of concentric rings of vascular and parenchyma tissue. Root size develops by increased cell multiplication and cell growth. A greater concentration of sucrose can be found in the smaller cells within the vascular region than in the larger cells of the parenchyma region (Milford, 1973). With the proportion of vascular to parenchyma cells fixed, this may explain the reason that breeders have only been able to develop sugar beet cultivars with the highest fresh weight concentration of sugar being around 18.0% (Cooke and Scott, 1993). Several hypotheses for this phenomenon have been stated, but no one knows for certain the reason why the parenchyma layer cells contain less sugar (Milford, 1973). One theory is that both vascular and parenchyma cells produce the same absolute amount of storage sugar, with the concentration thus being greater in the smaller (vascular) cells. Whatever the reason, data indicates that there is an inverse relationship between the weight of sugar beets produced per unit area and the percentage of sugar produced (Smith, 1987a). Recurrent and reciprocal recurrent selection techniques have not changed this inverse relationship between sucrose yield and root weight yield (Hecker, 1978).

Soon after new growth begins during the second growing season, the vernalized root bolts. Bolting occurs as the reproductive stage is initiated, with the plant producing an elongated stem, or tall angular seed stalk. A large petiolate leaf develops at the base of the stem with small leaves, less petiolate leaves and finally sessile leaves develop further up the stem. At the leaf axils secondary shoots develop forming a series of indeterminate racemes. These flowers are sessile and occur singly if monogerm, or in clusters, if multigerm (Smith, 1987a). The terms multigerm and monogerm refer to ripened fruit and not seed. Flowers are perfect including a tricarpellate pistil surrounded by the five stamens and a perianth of five sepals. The sugar beet flower contains no petals. Below and surrounding each flower is a slender green bract (Smith, 1987a).

Mature flowers begin anthesis about 5-6 weeks following the initiation of reproductive growth. Anthesis continues for a period of several weeks. Each fruit contains either a single seed or twin embryos (Smith, 1980). Pollination is carried out mainly by wind and, to a small degree, by insects (Cooke and Scott, 1993). The primary method of pollination is cross pollination because of the lack of synchrony between pollen release and receptiveness of the stigma (Cooke and Scott, 1993).

Approximately six weeks following full flower bloom, the seed crop is ready for harvest. Changes in color of the seedstalk and foliage, and shattering of the earliest maturing seeds are good indications of harvest time (Smith, 1980). The harvesting of seed of monogerm cultivars and those resistant to bolting is harder than the harvest of multigerm cultivars. In these cultivars the seed may be ready for harvest while the leaves and stalk are still green. In this case, seed development is the primary indicator of harvest readiness; i.e. when the seed is in the moderate to hard dough stage, then the crop is ready to harvest. (Smith, 1980).

C. Breeding of Sugar Beets

The genetic base of sugar beet germplasm is relatively limited since it originally was derived from only the variability expressed in the white fodder beets of Europe. It has been suggested by Bosemark (1979), that spontaneous hybridization with cultivated leaf-beet types and wild *B. vulgaris ssp. maritima* may have contributed additional variation. The genetic base of sugar beets remains narrow today even though the sugar beet is a cross pollinated crop (Panella, 1996). As will be discussed in this section, it is not easy to hybridize *Beta vulgaris* outside the *Beta* section.

Initial genetic research and plant breeding efforts in the U.S. were conducted at the end of the 19th Century by USDA scientists. Breeding efforts by private organizations began in 1910 and were dominant until 1925 when the USDA resumed breeding activity concentrating on development of strains resistant to the curly top virus and Cercospora Leaf Spot. Other breeding technologies developed by the USDA include cytoplasmic male sterility, monogerm seed and the concept of hybrid vigor (Panella, 1996). Today, private seed companies dominate sugar beet breeding concentrating on varieties which produce high sucrose yield, disease and pest resistance, and herbicide tolerance.

Cytoplasmic male sterility (CMS) allows the breeder to develop male-sterile or female parental lines. These lines are a key factor in the breeding of hybrid cultivars. CMS lines are the male sterile equivalents of O-type (or maintainer) lines. Commonly, a monogerm O-type of one line will be hybridized with the monogerm male-sterile equivalent of another line to produce a monogerm male-sterile F1. This F1 then is used as the seed parent in crosses with diploid or tetraploid pollinator lines.

In the U.S. all sugar beet cultivars are monogerm hybrids. The use of monogerm sugar beet seed has greatly reduced the major effort needed to thin clusters of sugar beet seedlings which were

the rule when multigerm seed was planted (Smith, 1987a). Monogerm seed was developed primarily through the work of displaced Russian scientist V. F. Savitsky. In 1950-52 he developed two monogerm lines, SLC 101 and SLC 107, and found that the monogerm trait is controlled by a single recessive gene labeled *mm* (Coons, Owen and Stewart, 1955).

In North America, most commercial hybrids are either diploid, triploid or anisiploid in nature. Three types of hybrids have been developed. These include single, double and three-way crosses. The three-way crosses have become the dominant hybrid cultivars in the U.S. These hybrids combine desired yield, beet quality and disease resistance attributes (Smith, 1987a; Hecker and Helmerick, 1985). Comparisons between equivalent diploid and triploid hybrids show similarity in yield of roots but, the triploids show less severe bolting (McFarlane, Skoyen and Lewellen, 1972). In a series of yield trials with diploid, triploid and tetraploid cultivars, the triploids produced the greatest level of sucrose. In this case, it was with the triploid which had the tetraploid as the female parent (Smith, Hecker and Martin, 1979).

The production and maintenance of inbred lines is an integral part of any sugar beet breeding program. This is carried out by bagging branches of mother roots. Sugar beets have a high level of self-incompatibility but there is a self-fertility gene which, when introduced, can create plants which are self-fertile (Smith, 1987a).

Sugar beet seed production must ensure the isolation of the flowering sugar beet plants from foreign pollen. The Oregon Seed Certification isolation distance between sugarbeets with different pollen sources is 3200 feet. The certification distance between sugarbeets and pollinators of other *Beta* species (i.e. fodder beet, red beet, Swiss chard) is 8000 feet. Most U.S. commercial sugar beet seed production is carried out in northwestern Oregon in the Willamette Valley. The climate in the Willamette Valley is close to ideal for producing sugar beet seed. Strip plantings are made of female plants (CMS) and male pollinator plants. Wider strips are planted of the CMS parent than the pollinator parent. The roots produced by these plants are allowed to overwinter. During the second season of growth, the reproductive stage takes over and seed is produced on the bolting stalks of these plants. The climate in Oregon is cold enough for vernalization to take place during the winter but normally not cold enough to kill sugarbeet roots. (Panella and Lewellen, 1998, Appendix VI).

Hybrids of sugar beet and *B. vulgaris ssp. maritima* are fertile and do not show incompatibility at the chromosome level (Panella and Lewellen, 1998). McFarlane, (1975) reported on hybrids involving *B. macrocarpa* and stated that they are rare due to different flowering dates of the species. Abe and colleagues (1986) reported pollen sterility and seed abortion in the F1 generation of crosses between *B. macrocarpa* and *B. vulgaris* and *B. vulgaris ssp. maritima*. In later generations segregation for chlorosis, hybrid weakness and sterility was observed, whereas distorted segregation occurred in the backcrossed progenies (Abe & Tsuda., 1988). Lange & De Bock, (1989) produced triploid and tetraploid hybrids between tetraploid *B. macrocarpa* and diploid or tetraploid cytotypes of *B vulgaris*. The triploids were nearly fully sterile, attributable to meiotic irregularities resulting from the triploidy. The tetraploid hybrids exhibited a somewhat better fertility. An F2 generation

showed partial hybrid dwarfness, partial fertility, as well as segregation for earliness and coloration of the hypocotyl.

Successful hybridizations between *B. vulgaris* and species in the section *Corollinae* have been carried out by several investigators. It is generally not necessary to use bridge species, although *B. vulgaris ssp. maritima* has been successfully used to introduce traits from *B. trigyna* into sugarbeet. Most of the hybrids with species of section *Corollinae* showed apomictic reproduction. The three species of section *Procumbentes* can be hybridized with *B. vulgaris* only with great difficulty. The hybrids become necrotic and die at the seedling stage. They either do no develop secondary roots, or they have poor vascular connection between the roots and the shoots. Such obstacles could be overcome by grafting the hybrids onto cultivated beet plants or by using fodder beets, mangels, *B. vulgaris ssp. maritima* as bridge species. The few surviving hybrids are highly sterile and only with difficulty can they be backcrossed with *B. vulgaris*. Pollen sterility in F1 and BC1 generations is the result of abnormal meiosis. Chromosome lagging, multiple spindles, bridges and ejected chromosomes have been frequently observed causing lack of fertility or embryo abortion. The chromosomes of the species of section *Procumbentes* do not pair with those of section *Beta* (Van Geyt et. al., 1990).

The development of the breeding of sugar beets has come a long way since its inception in the mid 1950's 1850's. At that time, the famous French plant breeder, Louis de Vilmorin, initiated the first real progress in this area by introducing the concept of progeny testing. (Coons, Owen and Stewart, 1955). In 1984, it was first reported that foreign genes were used to introduce new, desired genetic traits into plants. This was done via the use of *A. tumefaciens* transformation in which a plasmid, or vector, containing the foreign gene is introduced directly into the host plant's genome (Gasser and Fraley, 1989). Currently this and other plant transformation methods are being used to improve the sugar beet germplasm.

In the interim between de Vilmorin's early work and today's biotechnological approach, plant breeders have spent enormous effort in developing and attempting to develop resistant cultivars to the many viral, fungal and bacterial diseases of sugar beets. Due to the relatively narrow germplasm in sugar beet and its adaptation to many areas of the world, many new diseases and insect and nematode pests were encountered by this crop. Due to the inability to make many standard interspecific crosses in the genus *Beta*, biotechnology may offer a good deal of hope in developing disease, insect and nematode resistant cultivars. This topic was clearly defined as an important research area for sugar beet plant breeders (Panella, 1996).

D. Weediness Potential of Sugar Beets

It is well known that natural hybridizations between cultivated beet and some wild or weedy forms of section *Beta* can occur in areas where both are present. Boudry and colleagues demonstrated that cultivated sugar beet does outcross with weed populations of beet growing inland in Europe (Boudry et.al., 1993). He also concluded that the only increased risk of weediness herbicide tolerant sugar beet would pose over nontransgenic sugar beet is in sugar production areas

infested with weed beets, due to the fact that nonselective herbicides are routinely used to control weeds in these areas, and weed beets which have gained selectivity for a nonselective herbicide such as glufosinate-ammonium via pollen transfer with glufosinate-ammonium tolerant sugar beet would have an ecological fitness advantage over other populations of weed beets.

In North America the outcrossing potential of cultivated sugar beet with wild and/or weed beets which occur naturally is very different from that which exists in Europe. In North America the only wild or weed species of beet which exist in the same areas where cultivated sugar beet is grown are small, isolated populations of *B. macrocarpa* and *B. vulgaris ssp. maritima*, which is commonly referred to as the Milipitas beet which have been documented in California (see discussion below). A thorough discussion regarding the issue of gene flow from cultivated sugar beet to wild and/or weed beets, and specifically addresses the issue of herbicide tolerant sugar beet and its ecological impact, in the U.S. is provided by Dr. Lee Panella, USDA/ ARS, Fort Collins, CO, and Dr. Robert Lewellen, USDA/ARS, Salinas, CA. This discussion is included with this document in Appendix VI.

Small, longstanding, isolated populations of *B. macrocarpa*, in the Imperial Valley of California, and *B. vulgaris ssp. maritima*, in Santa Clara County, California, are the only known natural populations of weedy relatives of cultivated sugar beet occurring in sugar beet growing areas of North America (see discussion below). In the Red River Valley of North Dakota subzero weather during winter months does not allow for the carryover of viable seeds (USDA/APHIS, 1993). Many "wild" sugar beets are nothing more than escapes from commercial fields which grow in irrigation ditches (USDA/APHIS Permit 94-355-01, 1995). In its Environmental Assessment (EA) of a 1993 permit application, USDA/APHIS scientists stated that it is unlikely that descendants of crosses between transgenic and non-transgenic sugar beets would become part of breeding material or become established in any sugar beet ecological community (USDA/APHIS, 1993).

Some relatively small wild populations of *Beta* have become established in California due to the mild climate. These populations are described as *B. vulgaris ssp. maritima, B. macrocarpa,* and *B. vulgaris*. The populations of *B. vulgaris ssp. maritima* and *B. macrocarpa* are suggested to have developed from seed contaminants or from seed intentionally imported into California. The population of *B. vulgaris* is believed to have developed from sugarbeet itself. No wild populations of *Beta* have been reported in the U.S. outside of California (Panella and Lewellen, 1998, Appendix VI).

In 1928 Carsner reported wild beet populations in most of the older beet growing areas of California (McFarlane 1975). These beets were found in Imperial, Santa Clara, Ventura, San Bernardino, Los Angeles, and Orange Counties. Carsner speculated that these beets were either *B. vulgaris ssp. maritima* or hybrids between *B. vulgaris ssp. maritima* and sugarbeet. He was of the opinion that wild beets had been introduced into California as seed contaminants. With the exception of the wild populations noted above in Imperial and Santa Clara Counties there have been no further reports of these populations since Carsner. Information on Carsner's early find-

ings are reported by McFarlane, 1975.

However, McFarlane, (1975) identified the wild beets in Imperial County as *B. macrocarpa* rather than *B. vulgaris ssp. maritima*, as speculated previously by Carsner (1928). *Beta macrocarpa* is a species that occurs naturally in the Canary Islands and along the Mediterranean coastline. He speculated that seeds of *B. macrocarpa* were imported as contaminants in seed or in feed grain. McFarlane (1975), Abe (1988), and Panella and Lewellen (1998, Appendix VI), report that sugarbeet and *B. macrocarpa* do not readily produce viable hybrids.

Dahlberg and Brewbaker, (1948) referred to the population of *B. vulgaris ssp. maritima* in Santa Clara County as the "Milpitas wild beet". They speculate that this population 1) established itself from *B. vulgaris ssp. maritima* which was inadvertently imported from Europe along with sugar beet seed for the fledgling sugar beet industry, or 2) may have become established from seed brought in by the Franciscan Fathers when they established the Santa Clara and other missions in the late 1700's. The area where these beets were found is now highly urbanized and is no longer an area where sugar beets are commercially grown (Panella and Lewellen, 1998, Appendix VI).

Johnson and Burtch, (1959) reported the development of weed beets in California. They describe sugar beets which evolved into annual plants and became a weed problem. Recent surveys would indicate that such populations are restricted in size and appear to be localized in the Gilroy/Hollister area (Panella and Lewellen, 1998).

In summary it can be said that most of reports of wild beet populations in California were old reports with little or no follow-up study. The exception being the wild population of *B. macrocarpa* in Imperial County and the wild population of *B. vulgaris ssp. maritima* in Santa Clara County which are known to persist, the other populations having been eliminated (Panella and Lewellen, 1998). These populations have been established for many years and are small populations which have shown little propensity to spread. Commercial sugar beet is cultivated in Imperial County, and while pollen flow to the populations of *B. macrocarpa* in Imperial County could be argued, it has been documented that if hybrids were produced they would not survive in nature (Panella and Lewellen, 1998) . About 500 acres of commercial sugar beet are grown in Santa Clara County near Gilroy (USDA Census, 1992). This is approximately 20-25 miles from the small population of the so-called Milipitas beet which grows in the San Francisco Bay area.

As previously stated, sugar beet is a biennial crop and it is the second season's crop that produces seed for regeneration. However, certain conditions such as low temperatures after planting/emergence, low temperatures during the prior harvest season and longer day length can cause the sugar beet to "bolt" or produce a seed stalk during the first growing season (Bell, 1946; Jaggard, Wickens, Webb and Scott, 1983; Durrant and Jaggard, 1988). These situations exist in Europe, especially England when growers seed too early in the spring. They also can occur in California where sugar beets are often seeded during the fall and winter months or when spring planted crops are overwintered. Bolters are a problem in the current planted crop because, although inflorescences (bolting stalk) may be cut off, the bolted plants contain more lignin in their

roots and sugar yield could be reduced by 50% (Smith, 1987a; Scott and Wilcockson, 1976; and Jaggard, Wickens, Webb and Scott, 1983). While bolting can cause a problem in any given year it does not lead to any increase in weediness potential of sugar beet. Unwanted seed germination in sugar beet can be controlled by various methods such as hand pulling, or treatment with non-selective herbicides.

In a review of the State Noxious-Weed Seed Requirements Recognized in the Administration of the Federal Seed Act, no reference was found regarding "wild" sugar beets or wild sugar beet relatives as either restricted or noxious weeds. This demonstrates that sugar beet does not have the necessary attributes which could allow it to become a serious weed problem in sugar beet growing areas. There are wild species of sugar beets in the region surrounding the Mediterranean Sea and in the Caucasus Mountains of Russia and into Turkey and Iran. Wild species may also be found as far west as the Canary Islands. As discussed previously, no wild relatives exist in the U.S. except in a few locales in California (Doney, 1996; Cooke and Scott, 1993).

E. Potential for, and Consequences of Outcrossing

The potential for outcrossing in North America is provided in Appendix II, a 1995 letter from J. R. Stander, Betaseed, Inc., to USDA/APHIS providing rationale for moving the sugar beet authorization process from permit requirement to notification requirement, and in Appendix VI, a 1998 letter from Lee Panella, USDA/ARS and Robert Lewellen, USDA/ARS. There is very little risk of transgenic or non-transgenic sugar beets crossing naturally with wild relatives in the United States (see discussion in II.D). Crosses with species outside the *Beta* type are made, with difficulty, using special plant breeding techniques. In the report on the status of *Beta* Germplasm in the United States, four major areas for enhancement were pointed out. These include broadening genetic variability, pest resistance, gene transfer/molecular characterization and low tar genotypes (Panella, 1996). Due to the factors elucidated by Drs. Panella and Lewellen (Appendix VI) the risk of gene transfer from transgenic sugar beets involved in commercial production in the U.S.,is remote.

If a hybrid were to be created it would be easily removed either by the use of a herbicide other than glufosinate-ammonium, or mechanical means. Several classes of herbicides which are used to control broadleaf weeds also control sugar beet effectively. The sulfonyl-urea class of herbicides, the phenoxy class of herbicides, as well as dicamba will control sugar beet. Therefore it has been concluded by USDA experts in the area of sugar beet research such as Drs. Panella and Lewellen that herbicide tolerant sugar beet will not pose an increased risk of weediness potential over nontransgenic sugar beet, reference Appendix VI.

In the absence of herbicide treatment, viable offspring produced from gene pollen flow from glufosinate-ammonium tolerant sugar beet to weedy relatives would have no fitness enhancement over current populations of wild or weed beets which occur naturally in nonagricultural environments (Purrington and Bergelson, 1997).

III. The Transformation System and Plasmid Used

A. Transformation System

Transformation was performed at PLANTA Angewandte Pflanzengenetik und Biotechnologie GmbH, Einbeck, Germany. Calli of one individual plant of the pollinator R01, a sugar beet line in the third inbreeding generation, was transformed with the vector pOCA18/Ac using *A. tumefaciens* mediated transformation. A map of the plasmid is provided in Figure 1, on page 23. Calli cultures were incubated until secondary embryos had formed. Later, colonies were put into an enrichment medium containing kanamycin to selectively enrich for transformed colonies. Surviving colonies, selected for PAT expression, were then placed onto regeneration medium. Regenerated shoots were then transferred onto a rooting medium until normal plantlets appeared. Transformed plants were potted in a soilless mix and placed in a growth chamber. (PLANTA, 1997).

For transformation of plants the vector system as described by Olszewski et al. (1988). The vector system consists of an *Agrobacterium tumefaciens* strain and two plasmid components: 1) a nononcogenic Ti-plasmid and 2) a binary cloning vector based on the plasmid pOCA18/Ac. The nononcogenic Ti-plasmid, from which the T-region has been deleted, carries the vir genes required for transfer of T-DNA present on the second plasmid to the plant genome. On the binary vector, the gene of interest - e.g. the chimeric *pat* gene - is located between the T-DNA border sequences.

B. Parent Tissue Culture Line

Tissue culture line R01, a sugar beet line in the third inbreeding generation, was transformed with the vector pOCA18/Ac using *A. tumefaciens* mediated transformation. To produce a line of 100% homozygoucity for tolerance to glufosinate-ammonium, the transformed plant, now referred to as event T120-7, was selfed. The resulting lines, 1022S, 1026S and 1031S are sublines of the original transformed R01 line. The parent line (R01) as well as the transformed lines resulting from transformation event T120-7 are fully fertile.

C. Construction of the Plasmid Used for Transformation

Construction of the vector pOCA18/Ac is described in detail in Olszewski et al. (1988). The backbone of this plasmid is the broad host range vector pRK290 (Ditta et al. (1980). Additional elements contained in the vector are given in Table 2. To obtain pOCA18/Ac the synthetic *pat* gene from *Streptomyces viridochromogenes* fused to promoter and terminator of Cauliflower Mosaic virus (CaMV) was inserted as a SalI fragment into the T-DNA. Details about the nononcogenic Ti-plasmid component are given in Olszewski et al. (1988).

D. Open reading Frames and Associated Regulatory Regions in pOCA18/Ac

1. CaMV 35 S Promoter and Terminator

The 35S promoter and terminator sequences are derived from CaMV and control expression of the *pat* gene. CaMV is a doublestranded DNA caulimovirus with a host range restricted primarily to cruciferous plants. The region of the CaMV genome used corresponds to nucleotides 6909 - 7437 for the promoter and nucleotides 7439 - 7632 for the terminator (Pietrzak et al., 1986). The 35S promoter directs high level constitutive expression in higher plants and is widely used as a promoter for high expression of genes (Harpster et al., 1988). The CaMV sequences, as used in glufosinate-ammonium tolerant sugar beet do not cause the sugar beet to become a plant pest.

2. pat

The pat gene is a synthetic version of the *pat* gene isolated from *Streptomyces viridochromogenes*, strain Tü 494 (Bayer et al., 1972). It encodes the enzyme phosphinothricin acetyltransferase (PAT), which imparts resistance to the phytotoxic activity of glufosinate-ammonium. Since the native *pat* gene has a high G:C content, which is atypical for plants, a modified nucleotide sequence was synthesized using codons preferred by plants. The amino acid sequence of the enzyme remains unchanged. The nucleotide sequences of the native and synthetic gene share 70% homology. Genes encoding PAT enzymes have been isolated from *S. viridochromogenes* (Hara et al., 1991) and *S. hygroscopicus* (Thompson et al., 1987).

3. aph (3') II

The gene for aminoglycoside (3') phosphotransferase type II (APH(3') II) was first isolated from transposon Tn5 which has its natural environment in *Escherichia coli* (Beck et al., 1982). *aph (3') II* is also known as *npt II*. Expression of APH (3') II mediates resistance to aminoglycoside antibiotics (neomycin, kanamycin). This gene is often used as a marker gene in genetic modification experiments (Horsch et al., 1984).

Figure 1: Plasmid Map of the Vector pOCA18/Ac

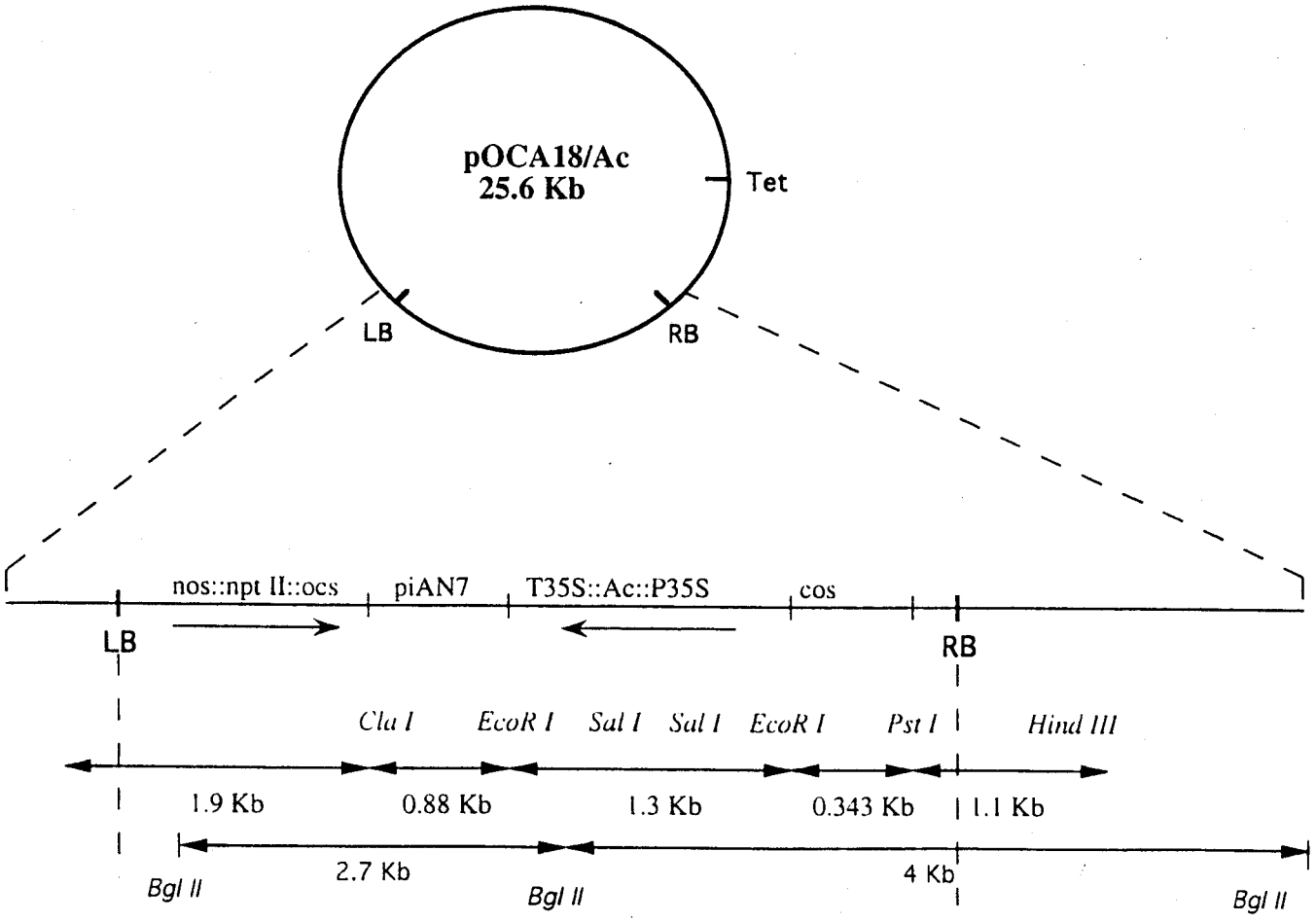
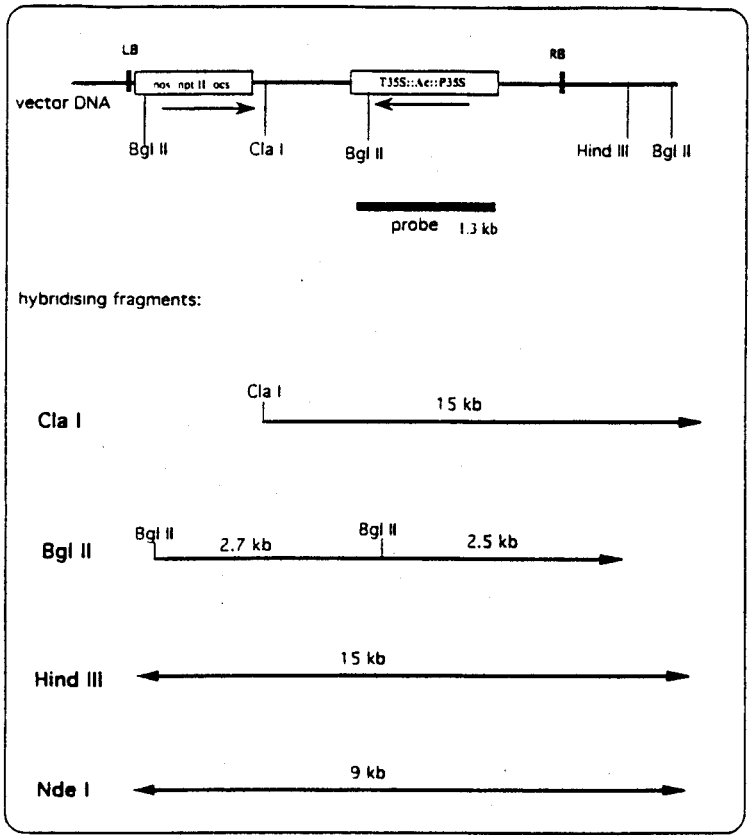


Figure 2: Southern Analysis of Event T120-7, Insert Characterization, Panel A; and Stability, Panel B

A.



B.

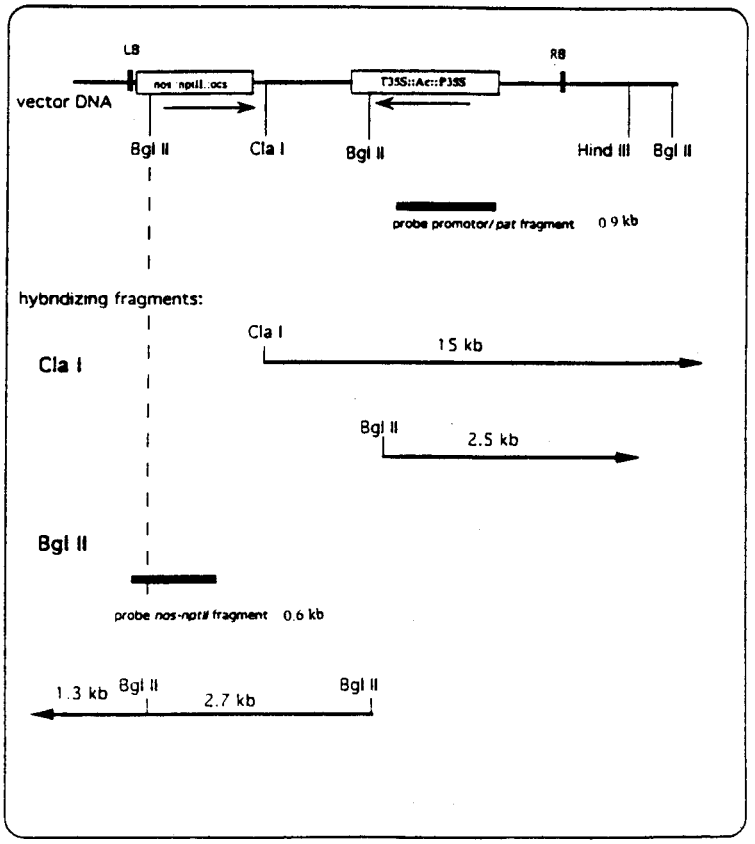


TABLE 2: Description of the Genetic Elements of the Vector pOCA18/Ac:

Genetic Element	Position in Vector	Size (Kb)	Function
RB	1 - 903	903	derived from ***A. tumefaciens*** pTi37 Ti-plasmid (including Right Border) (Depicker A. et al, J. Mol. Appl. Genet.1, (1982), pp561-573)
cos	904 - 1250	346	cos site from bacteriophage Lambda (Feiss and Campbell, J. Mol. Biol. 83, (1974), pp527-540)
	1251 - 1273		synthetic polylinker sequences
P-35S	1274 - 1805	531	35S promoter from Cauliflower Mosaic Virus from the vector pDH51(Pietrzak M. et al., Nucleic Acids Res. 14, (1986), pp. 5857-5868)
	1806 - 1830		synthetic polylinker sequences
pat	1831 - 2382	551	synthetic *pat* gene (amino acid sequence from *Streptomyces viridochromogenes*) (Strauch et al. (1993) European patent 275957 B1))
T-35S	2383 - 2608	225	35S terminator from Cauliflower Mosaic Virus from the vector pDH51(Pietrzak M. et al., Nucleic Acids Res. 14, (1986), pp. 5857-5868)
ori	2609 - 3463	854	derived from synthetic ***E.coli*** vector PiAN7 including ori ColE1 (Huang et al., Biotechnology 10, (1988), pp. 269-283
T-*ocs*	3464 - 4256	792	terminator of the *octopine synthase* gene (De Greve et al. J. Mol. Appl. Genet.1, (1982), pp 499-511) and (Gielen J. et al., (1984), EMBO J. 3; pp. 835-846)
aph (3') II (npt II)	4256 - 5051	795	*aminoglycoside (3') phosphotransferase type II (neomycinphosphotransferase II)* gene from *E. coli* transposon Tn5 (Beck et al Gene 19, (1982), pp 327-336)
P-*nos*	5052 - 5389	337	promoter of the *nopaline synthase* gene (Depicker A. et al, J. Mol. Appl. Genet.1, (1982), pp561-573)
LB	5390 - 5414	24	synthetic T-DNA Left Border from *A. tumefaciens* Ti plasmid Ach5 (Gielen J. et al., (1984), EMBO J. 3; pp. 835-846)

IV. Molecular Characterization of Transformation Event T120-7

A. A. Description, History and Mendelian Inheritance of Event T120-7

The primary transformation event T120-7 is derived from the transformation of tissue culture line R01 as described in Section III. Through traditional breeding with these fertile transformation events, individuals homozygous at the *pat* locus have been produced. These have been crossed with both commercially available public inbred lines and proprietary inbred lines. Traditional backcrossing and breeding will be used to continue to transfer the glufosinate-ammonium resistance locus in event T120-7 to a wide range of sugar beet varieties.

Transformation event T120-7 has been field tested by AgrEvo USA Company since 1994 in the primary sugar beet growing regions of the United States. These tests have been conducted at approximately 68 sites under field release authorizations granted by APHIS (USDA authorizations: 94-347-01r; 96-052-02r; and, 97-029-01r). Transformation event T120-7 has also been field tested extensively in Europe, including Germany, Great Britain, France, and the Former Soviet Union. T120-7 has also been field tested in Canada. The great majority of the trials have been efficacy trials in which the plants have been sprayed with different rates of glufosinate-ammonium. When sprayed with the herbicide, plants, in trials worldwide, exhibited a tolerance to glufosinate-ammonium tolerance, indicating that the gene is stably integrated and expressed.

The *pat* locus has been stabilized in T120-7 homozygotes for several generations. To incorporate this transformation event the original hemizygous transformed plants were self-pollinated. Glufosinate-ammonium tolerant plants were backcrossed to the non-transgenic line creating BC1. Selfing of these plants resulted in Back Cross 1(BC1) progeny segregating in a 3:1 fashion with respect to glufosinate-ammonium tolerance (see Table 3). Resistant progeny expressing tolerance were selected from a population of young sugar beet plants by spraying with glufosinate-ammonium. These homozygous or hemizygous individuals were again self-pollinated producing progeny which segregated with respect to glufosinate-ammonium tolerance. The resistant progeny were either homozygous or hemizygous for the *pat* locus. Homozygous plants were those from which all progeny from the 2nd self-pollination were unharmed by glufosinate-ammonium. The seed from the homozygous plants were backcrossed to the nontransgenic pollinator line and the Back Cross 2 (BC2) progeny were sprayed with glufosinate-ammonium. If the *pat* locus is stable, then 50% of the progeny should be resistant to glufosinate-ammonium. This indeed was the case (see Table 3 for results). The genetic segregation ratios obtained from BC1 and BC2 populations do not deviate greatly from the ratios that would be expected from the assumption that the herbicide resistant gene is inherited in a Mendelian fashion as a single dominant gene. Further evidence supporting stable integration is shown by Southern blot analysis of four different progenies of different years of T120-7 (See Section IV.B.3).

Table 3. Segregation Data Regarding Sugar beet Transformation Event T120-7

Cross	total plants	Resistant	Sensitive	χ2 value[b]
BC1[a] (expected)	104	78	26	0.8205
BC1 (observed)	104	74	30	
BC2[a] (expected)	236	118	118	3.8136
BC2 (observed)	236	103	133	

[a] BC1 and BC2 indicate Backcross generation 1 and Backcross generation 2, as described in the text. For BC1, 75% of the plants were expected to be resistant. For BC2, 50% of the plants were expected to be resistant.

[b]No significant difference ($p = 0.05$) for the χ^2 goodness -of-fit test (significance at $p = 0.05$ for $\chi^2 > 3.84$, $df = 1$).

B. DNA Analysis of Glufosinate-Ammonium Tolerant Sugar Beet

To determine the nature, number and molecular stability of insertions which occurred in transformation event T120-7, Southern hybridization and PCR analysis were used. Southern analysis was used to determine the copy number of the insertions and the stability of these insertions over several generations. Both Southern and PCR analyses were used to map the inserted DNA.

1. Verification of Insert Integrity and Copy Number

When transforming a plant with *Agrobacterium* it is not certain whether only the T-DNA portion of the binary vector will transfer to the plant's genome, and there is no way to predict how many copies of the T-DNA will integrate. We have therefore used a combination of Southern blot and PCR analyses to examine the integrity of the inserted T-DNA in transformation event T120-7. These analyses also indicate the T-DNA copy number.

a. Southern blot analysis

The DNA from progeny of transformation event T120-7 was isolated and digested with several enzymes (Bgl II, Cla I, Hind III or Nde I) (Kraus, J., 1997). See Figure 2 to locate restriction sites in pOCA18/Ac. After separation of the DNA by electrophoresis on agarose gels, the DNA was transferred to a nylon membrane and hybridized with a ^{32}P-labeled fragment including the *35*S promoter::*pat*::*35*S terminator cassette (1.3 kb EcoRI fragment) or with a ^{32}P-labeled fragment containing the *nos* promoter and part of the *aph (3') II* gene sequence (0.6 kb fragment).

The Southern blots are shown in Figures 3 and 4, on pages 29 and 30, respectively; and in Figures 5a, 5b and 5c, on page 31 (control blots). The hybridizing fragments expected and observed when using the described probes are listed in Table 4.

Table 4. Hybridizing Fragments in Southern Blots of T120-7 DNA Probed with the *35*S promoter::*pat*::*35*S terminator or *nos* promoter::*aph(3')II* Sequences

Restriction Enzyme	*35*S promoter::*pat*::*35*S terminator probe		*nos* promoter::*aph(3')II* probe	
	Expected Fragment (kb)[a]	Observed Fragment (kb)	Expected Fragment (kb)[a]	Observed Fragment (kb)
Bgl II	2.7, 1 unknown	2.7, 2.5	2.7, 1 unknown	2.7, 1.3
Cla I	1 unknown	15	1 unknown	ND [b]
Hind III[c]	1 unknown	15	1 unknown	ND
Nde I[d]	1 unknown	9	1 unknown	ND

[a] Expected fragment sizes for 1 copy of inserted vector.
[b] ND = not done
[c] Hind III does not cleave within the T-DNA (within the left and right borders)
[d] Nde I does not cleave within the plasmid DNA. See Figure 2 for restriction enzyme locations.

The sizes of some hybridizing fragments can be predicted by the location of restriction enzyme cleavage sites internal to the inserted T-DNA. Those hybridizing fragments whose sizes cannot be predicted result from cleavage in the integrated T-DNA and/or in the adjacent plant DNA.

In Figure 3 on page 29, the T120-7 DNA was probed with a fragment including the *35*S promoter::*pat*::*35*S terminator cassette. Digestion with Bgl II (lane 1) gives 2 hybridizing fragments. The 2.7 kb fragment is internal to the T-DNA; the 2.5 kb fragment results from cleavage in the integrated T-DNA and in the adjacent plant DNA. This single additional band is evidence that only one copy of the T-DNA, and hence the *pat* gene, has inserted into the plant genome. Only one hybridizing fragment is detected in the Nde I (lane 5), Cla I (lane 9), and Hind III (lane 13) digests, as expected. The single hybridizing fragment in the Cla I (lane 9) digest results from cleavage of the restriction site in the T-DNA and in the adjacent plant DNA. The single hybridizing fragment in the Nde I (lane 5) and Hind III (lane 13) digests results from cleavage in the adjacent plant DNA only. These data provide good evidence that only one copy of the T-DNA from pOCA18/Ac was integrated into the plant genome in transformation event T120-7.

In Figure 4 on page 30, the T120-7 DNA was probed with a fragment containing the *nos* promoter and part of the *aph (3') II* gene fragment. Digestion with Bgl II gives 2 hybridizing fragments. The 2.7 kb fragment is internal to the T-DNA; the 1.3 kb fragment results from cleavage in the integrated T-DNA and in the adjacent plant DNA. This single additional band is evidence that only one copy of the T-DNA, and hence the *aph(3')II* gene, has inserted into the plant genome. This data confirms the result found when a fragment including the *35*S promoter::*pat*::*35*S terminator cassette was used as a probe.

Figure 3: Southern Analysis of Event T120-7 probed with a fragment including the *35S* promoter::*pat*::*35S* terminator cassette. DNA (10 µg) were digested with the following restriction enzymes: Bgl II (lanes 1-4) Nde I (lanes 5-8), Cla I (lanes 9-12) and Hind III (lanes 13-15). T 120-7 DNA is contained in lanes 1, 5, 9 and 13. Nontransgenic sugar beet DNA is contained in lanes 4, 8 and 12. Bacteriophage λ DNA digested with pst I was used as the size marker. A 1.3 kb EcoRI fragment including the *35S* Promoter::*pat*::*35S* Terminator Cassette was used as a probe.

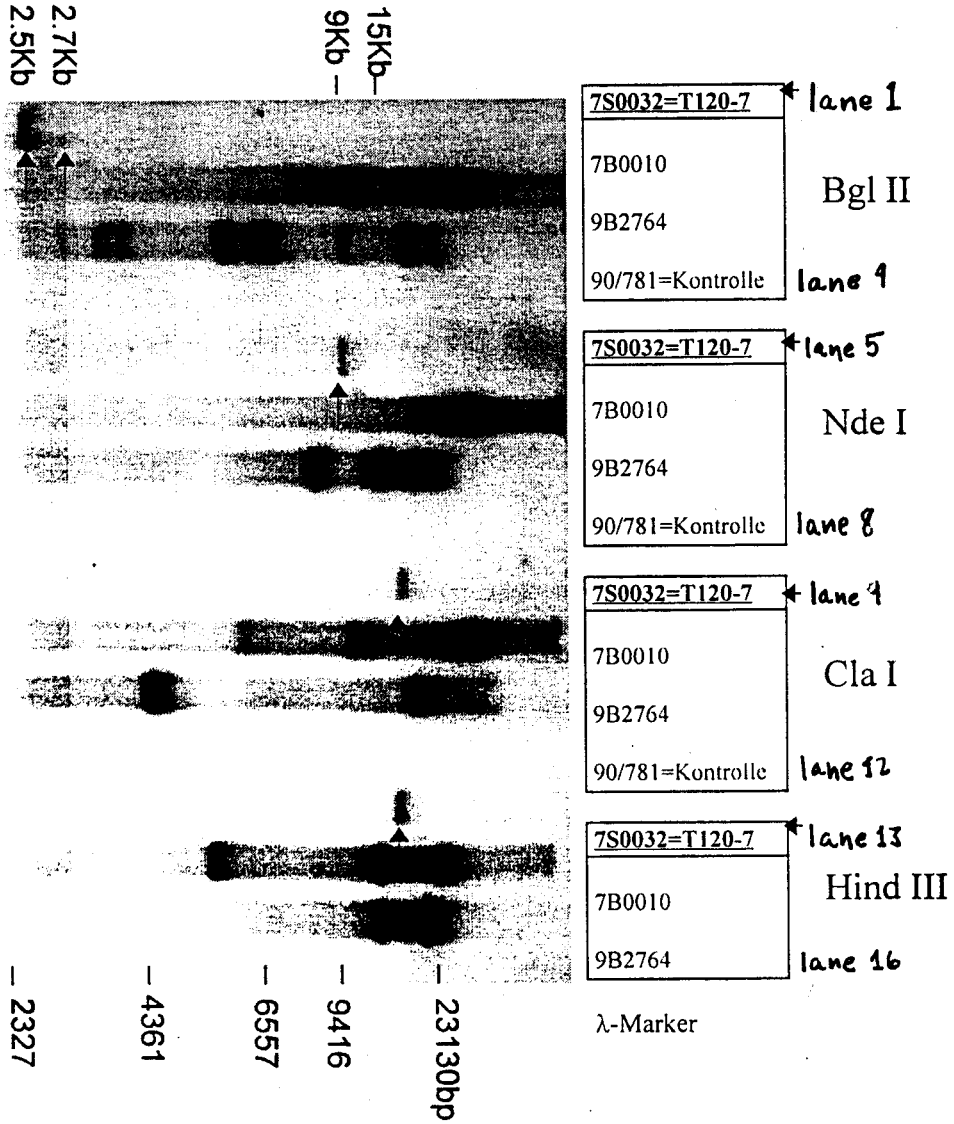


Figure 3 Legend:
lane 1: T120-7 DNA digested with Bgl II
lane 5: T120-7 DNA digested with Nde I
lane 9: T120-7 DNA digested with Cla I
lane 13: T120-7 DNA digested with Hind III
(The only lanes of interest are 1, 5, 9 and 13 as they contain T120-7 data. The other lanes contain data for sugar beet lines not relevant to this petition document.)

Figure 4: Southern Analysis of Event T120-7 probed with a fragment containing the *nos* promoter and part of the *aph (3') II* gene sequence. The DNA from T120-7, a nontransgenic counterpart (negative control) and four (4) progenies of T120-7 (1015, 1031, 1026 and 1022) were digested with Bgl II prior to electrophoresis. The blot was hybridized with a fragment containing the *nos* promoter and part of the *aph (3') II* gene sequence (0.6 kb). Raoul markers were used as the size markers.

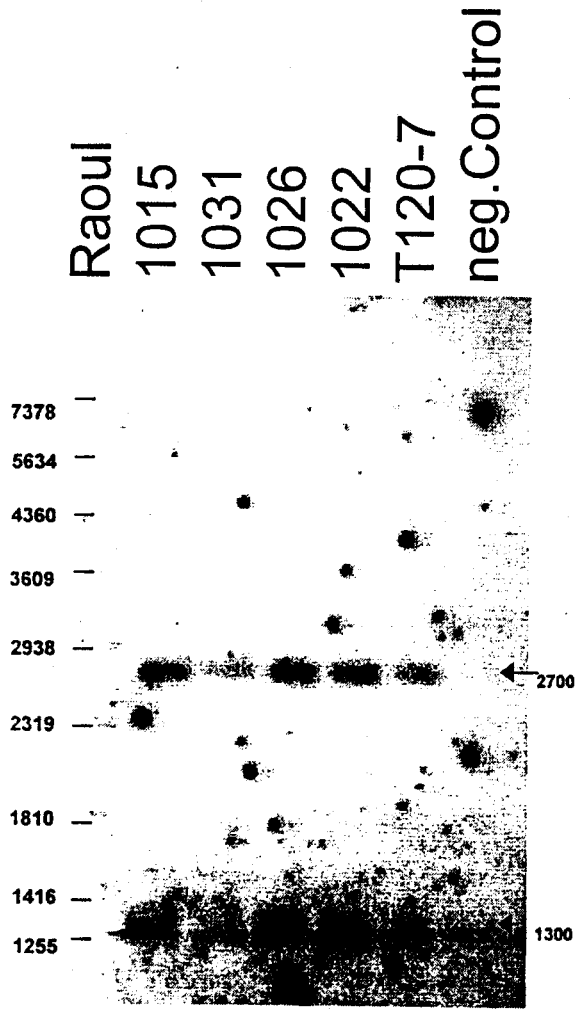


Figure 4 Legend:
Raoul: Molecular weight marker ladder
1015: progeny 1015 DNA
1031: progeny 1031 DNA
1026: progeny 1026 DNA
1022: progeny 1022 DNA
T120-7: original transformant plant DNA
Neg. Control: nontransgenic counterpart DNA

Figure 5: Southern Analysis of Plasmid DNA and Nontransgenic Sugar beet . In Panel A, DNA (10 µg) was digested with one of the restriction enzymes BglII, NdeI, ClaI or HindIII, respectively, prior to electrophoresis. The blot was hybridized with the *nos- (aph (3') II) nptII* (0.6 kb)sequences. In Panel B, DNA (10 µg) was digested with one of the restriction enzymes BglII, NdeI, ClaI or HindIII, respectively, prior to electrophoresis. The blot was hybridized with the P-35S/*pat*/T-35S (1.3 kb) sequences. In Panel C, plasmid DNA (10 µg) was digested with one of the restriction enzymes BglII, NdeI, ClaI or HindIII, respectively, prior to electrophoresis. The blot was hybridized with the 35S:: *pat* (0.9 kb) sequences.

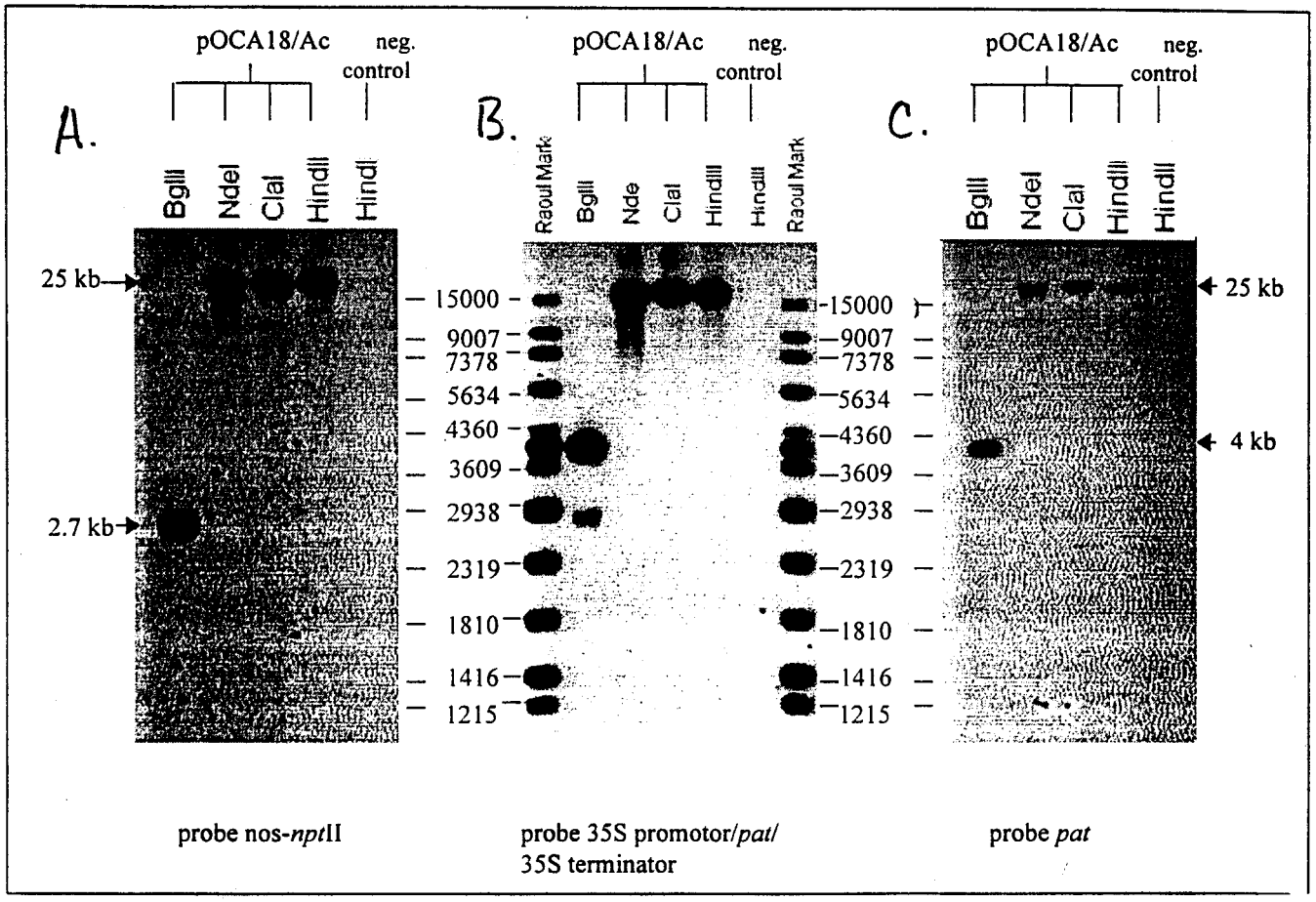


Figure 5a Legend:	Figure 5b Legend:	Figure 5c Legend:
BglII: transforming plasmid DNA digested with BglII	**Raoul Mark:** Molecular weight marker Raoul	**BglII:** transforming plasmid DNA digested with BglII
NdeI: transforming plasmid DNA digested with NdeI	**BglII:** transforming plasmid DNA digested with BglII	**NdeI:** transforming plasmid DNA digested with NdeI
ClaI: transforming plasmid DNA digested with ClaI	**NdeI:** transforming plasmid DNA digested with NdeI	**ClaI:** transforming plasmid DNA digested with ClaI
HindIII: transforming plasmid DNA digested with HindIII	**ClaI:** transforming plasmid DNA digested with ClaI	**HindIII:** transforming plasmid DNA digested with HindIII
HindIII, neg. control: nontransgenic sugar beet DNA digested with HindIII	**HindIII:** transforming plasmid DNA digested with HindIII	**HindIII, neg. control:** nontransgenic sugar beet DNA digested with HindIII
	HindIII, neg. control: nontransgenic sugar beet DNA digested with HindIII	
	Raoul Mark: Molecular weight marker Raoul	

The Figures 3 (lanes 4, 8, 12) and 4 (negative control lane) as well as Figures 5a, 5b and 5c (control blot), show that the probes were specific to the introduced sequences in event T120-7 since no hybridization was seen with nontransgenic sugar beet DNA. Hybridization of restricted pOCA18/Ac DNA with the probes verify that they identify the target sequences (Figure 5, control blot). In summary, Southern blot analyses show event T120-7 contains one T-DNA insert and, hence, one copy of the *pat* and *aph(3')II* genes.

b. PCR analysis

The DNA from transformation event T120-7 was isolated and subjected to PCR analysis along with pOCA18/Ac vector DNA (Kraus, J., 1997). The purpose of this experiment was to show that sequences outside the Ti-plasmid borders of pOCA18/Ac did not integrate into the genome of T120-7. For these experiments seven different primer pairs were used. PCR products were separated on agarose gels and stained with ethidium bromide. The location of primers on the vector and the PCR products expected with vector DNA as the template are shown in Figure 6, page 33. Event T120-7 DNA mixed with pOCA18/Ac DNA was used as a positive control for the function of the primers. The gel of PCR products when sugar beet DNA +/- vector DNA was the template are shown in Figure 7 page 34. The data are not shown for nontransgenic sugar beet DNA as no products were observed.

All controls, plant DNA mixed with vector DNA (lanes 2, 4, 6, 8, 10, 12, 14), show a PCR fragment of the expected size, indicating that all primers function properly. The *aph(3')II* and *pat* genes are detectable in DNA from T120-7 (lanes 13 and 15, respectively), confirming the Southern data. The products expected with primer pairs homologous to sequences inside the left or right Ti-plasmid borders are amplified (lanes 5 and 9, respectively). However, using one primer located oustide the left (lane 3) and the right (lane 7) borders does not result in a product. Additionally, there is no product formed when using a primer pair specific to the tet^R gene (lane 11). The results from these last three primer pairs indicate that sequences outside the T-DNA have not integrated into the genome of event T120-7.

In conclusion, Southern and PCR analyses indicate that transformation event T120-7 and its progeny contain 1 copy of the T-DNA from vector pOCA18/Ac. Furthermore, no DNA from outside the T-DNA borders has integrated into the genome. It can be concluded that event T120-7 contains 1 copy of the *pat* and *aph (3') II* genes.

Figure 6: Primer Products and Location of Primers used for PCR Analysis of Event T120-7. Primer locations are indicated with small arrows. Expected product sizes are given for primer pairs when the T120-7 DNA (P) is used alone or with vector pOCA 18/Ac DNA (P+V).

DNA	primer combination	expected size of PCR fragment (in bp)
lane1 : marker		
lane2 : P+V	ocs-ter/ oLB	1905
lane3 : P	ocs-ter/ oLB	----
lane4 : P+V	ocs-ter/ iLB	1417
lane5 : P	ocs-ter/ iLB	1417
lane6 : P+V	35S-ter/ oRB	1667
lane7 : P	35S-ter/ oRB	----
lane8 : P+V	35S-ter/ iRB	1394
lane9 : p	35S-ter/ iRB	1394
lane10 : P+V	tet1/ tet2	768
lane11: P	tet1/ tet2	----
lane12: P+V	npt II 1/ npt II 2	550
lane13: P	npt II 1/ npt II 2	550
lane14: P+V	pat 1/ pat 2	907
lane15: p	pat 1/ pat 2	907

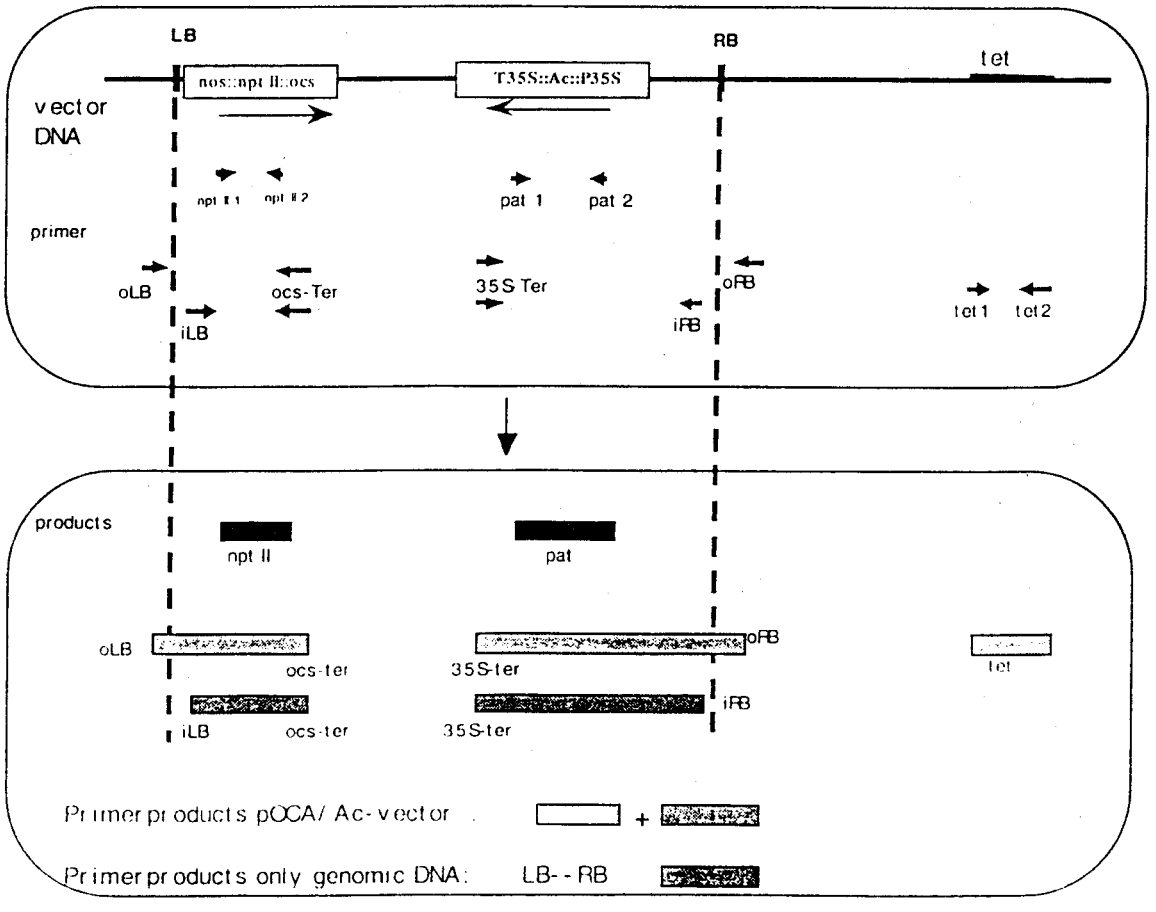


Figure 7: PCR Analysis of Event T120-7. Primer pairs were used to generate products from T120-7 DNA in combination with vector pOCA18/Ac DNA (lanes 2, 4, 6, 8, 10, 12 and 14), or from T120-7 DNA alone (lanes 3, 5, 7, 9, 11, 13, and 15). See Figure 6, page 34, for a description of Primer pairs. Raoul Markers were used as the size markers (lane 1).

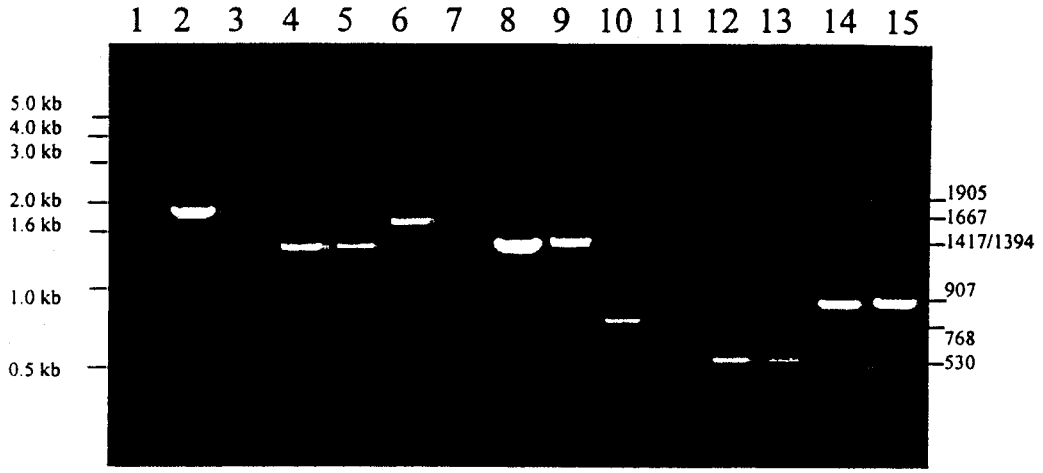


Figure 7Legend:
lane 1: Kb marker ladder
lane 2: plant DNA + plasmid (pOCA18/Ac); ocs-ter/oLB
lane 3: plant DNA; ocs-ter/oLB
lane 4: plant DNA + plasmid (pOCA18/Ac); ocs-ter/iLB
lane 5: plant DNA; ocs-ter/iLB
lane 6: plant DNA + plasmid (pOCA18/Ac); 35S-ter/oRB
lane 7: plant DNA; 35S-ter/oRB
lane 8: plant DNA + plasmid (pOCA18/Ac); 35S-ter/iRB
lane 9: plant DNA; 35S-ter/iRB
lane 10: plant DNA + plasmid (pOCA18/Ac); tet1/tet2
lane 11: plant DNA; tet1/tet2
lane 12: plant DNA + plasmid (pOCA18/Ac); nptII 1/nptII 2
lane 13: plant DNA; nptII 1/nptII 2
lane 14: plant DNA + plasmid (pOCA18/Ac); pat 1/pat 2
lane 15: plant DNA; pat 1/pat 2

3. Stability of Insertions

The Southern and PCR data indicate that there is one copy of the T-DNA from binary vector pOCA18/Ac present in the genome of transformation event T120-7 (Kraus, J., 1997). To demonstrate stability of the integrated DNA over several generations, the original transformation event, T120-7, was compared with four (4) progenies (Kraus, J., 1997). The progenies were produced in 1994 (1015, 1013) and 1995 (1026, 1022), and are a result of self-pollinations or crosses with nontransgenic sugar beet lines. For these analyses genomic DNA was digested with either Bgl II or Cla I. After transfer to a nylon membrane the DNA was hybridized with a ^{32}P-labeled *35*S promoter::*pat* fragment (0.9 kb) (Figures 8a and 8b, on page 36), or with a ^{32}P-labeled fragment containing the *nos* promoter and part of the *aph (3') II* gene fragment (0.6 kb fragment) (Figure 4, page 30). The autoradiographs of the blots show that the integration pattern is unchanged for the generations and progenies observed, thus demonstrating stability of the inserted sequences and traits. Segregation data (Section IV.A) further confirm the stability of the inserts, and show that they segregate as one dominant Mendelian locus. See Figures 8a, 8b and 4, on pages 36 and 30, respectively; and, Figure 5 (control blot), page 31, for verification that the probes identify the appropriate target sequences.

Figure 8: Southern Analysis of T120-7 DNA to characterize insert stability. Panel A: Plant DNA digested with Restriction Enzyme Cla I and hybridized with *35S* promoter:*pat* fragment. Panel B: Plant DNA digested with Restriction Enzyme Bgl II and hybridized with *35S* promoter:*pat* fragment.

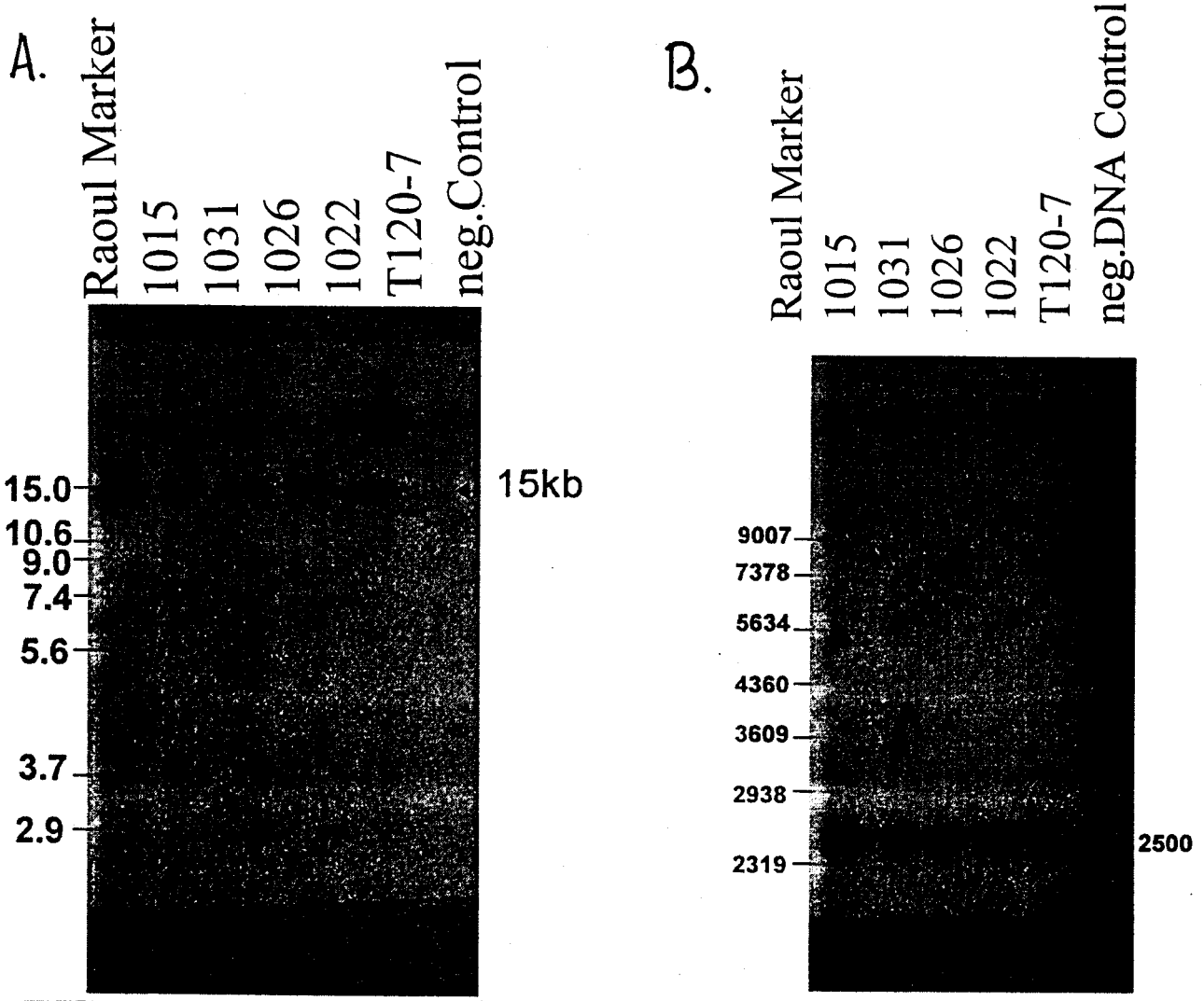


Figure 8a Legend:	**Figure 8b Legend:**
Raoul: Molecular weight marker ladder	**Raoul:** Molecular weight marker ladder
1015: progeny 1015 DNA	**1015:** progeny 1015 DNA
1031: progeny 1031 DNA	**1031:** progeny 1031 DNA
1026: progeny 1026 DNA	**1026:** progeny 1026 DNA
1022: progeny 1022 DNA	**1022:** progeny 1022 DNA
T120-7: original transformant plant DNA	**T120-7:** original transformant plant DNA
Neg. Control: nontransgenic counterpart DNA	**Neg. Control:** nontransgenic counterpart DNA

C. Gene Expression in Sugar Beet Event T120-7

The levels of PAT and APH(3')II proteins in transformation event T120-7 and nontransgenic counterparts were determined in beet tops and roots by Enzyme Linked Immunosorbent Assay (ELISA) (Shillito, 1997). The ELISA assays use polyclonal antibodies which detect both degraded and intact PAT or APH (3')II enzyme. Therefore, the enzyme detected may not all be functional. Both assays are the sandwich immunoassay type in which PAT or APH (3') II antibodies are used to coat the wells. Samples consisting of transformant extracts, non-transformant extracts as controls, and pure PAT or APH (3') II protein as a standard are added to the wells. ELISA assays were performed on field grown sugar beet plants harvested at maturity from 6 US field sites. Results from the ELISAs are shown in Table 5. Thorough explanations of both the PAT and APH(3')II ELISA methodologies are given in Appendix VII, the test kit instructions. Although both the PAT and APH (3') II enzymes were detected in sugar beet plant matricies, it has been determined that neither PAT, nor APH (3') II is active when pulp or molasses are fed to animals. It has been determined that PAT is rendered inactive during processing due to the temperatures reached (Shultz et al., 1997).

Table 5. Quantities of PAT and APH(3')II in Sugar Beet Plants (T120-7 progeny) as Detected by ELISA

Plant Matrix[c]	% Protein[a]	ng PAT/ g protein[b,d]	ng APH (3') II/ g protein[b,d]
roots	6.8	137	23
tops	15.0	966	51
pulp (dried)	9.7	n.d.	1.6
molasses	9.9	n.d.	n.d.

[a] Accepted literature values (Ensminger et al., 1990) used for calculation purposes. Values reported are moisture-free.
[b] Two extracts from each sample (18 tops; 18 roots from 6 field sites) were analyzed in triplicate.
[c] controls: Positive control, nontransgenic sugar beet from same site fortified with pure PAT or APH(3')II protein. Negative control, nontransgenic sugar beet from the same site. Values reported are mean values from all sites.
[d] Limit of Detection (LOD), PAT = 2 ng/g sample (root), 1.6 ng/g sample (sugar, pulp), 0.4 ng/g (molasses); LOD, APH(3')II = 0.35 ng/g sample (root, pulp and molasses); n.d.: not detected

The data in Table 5. indicate that a small amount of PAT as well as APH (3') II protein is present relative to total protein in the tops. Less than 1% of sugar beet tops are used as cattle feed (Reference Appendix IV). No PAT protein was detected in pulp or molasses, both of which are used in cattle feed. A minute amount of APH (3') II was detected in pulp. No PAT or APH (3') II enzyme was detected in nontransgenic genetic counterparts of transformation event T120-7.

V. *Agronomic Performance and Compositional Analysis of Glufosinate-Ammonium Tolerant Sugar beet*

A. Field Tests and Agronomic Characteristics

Field tests with Event T120-7 sugar beet have been carried out in the primary sugar beet growing regions of the USA since 1994. In total, 68 trials have been conducted. In 1994, three (3) trials were conducted under USDA authorization 94-054-06r; in 1995 six (6) trials were conducted under USDA authorization 94-347-01r; in 1996 nineteen (19) trials were conducted under USDA authorization 96-052-02r; and, in 1997 forty-four (44) trials were conducted under USDA authorization 97-029-01r. Complete field report data for each year is given in Appendix 1. In all trials over the four years no differences were reported regarding insect susceptibility and disease resistance in the transformed sugar beet versus a nontransgenic counterpart beet. In all tests crop tolerance to the herbicide was excellent; weed control was good to excellent. No differences in T120-7 sugar beet compared to nontransformed counterpart beets as well as standard commercial sugar beet varieties growing in nearby fields were measured in the agronomic characteristics plant emergence, seedling vigor, and stand establishment. In comparative analyses, the transgenic sugar beet was within accepted values as commercial sugar beet varieties with regards to root size and weight, sugar yield (corrected), sugar content, and various other minerals. Event T120-7 has also been field tested extensively in Western and Eastern Europe, and in the Former Soviet Union. Event T120-7 has also been field tested in Canada.

B. Disease and Pest Characteristics

Sugarbeets are susceptible to damage by a variety of viral, bacterial and fungal pathogens as well as being subject to attack by various insect and nematode pests. Important diseases of sugar beet are Cercospora Leaf Spot (***Cercospora beticola)***, Rhizoctonia Root Rot (***Rhizoctonia solani)***, Beet Curly Top, Rhizomania (beet necrotic yellow vein virus), and Virus Yellows. Insect pests include the Sugarbeet Root Maggot (***Tetanops myopaeformis)***, and the Sugarbeet Root Aphid (***Pemphigus populivenae (betae))*** . Important plant parasitic nematodes which can be pests of sugarbeet are the Sugarbeet Cyst (***Heterodera schachtii)***, Root Knot (***Meloidogyne arenaria, M. incognita, M. javanica, and M. hapla)***, and False Root Knot nematodes (***Nacobbus aberrans,*** and ***N. dorsalis)***. (Reference Appendix 5 for more information on diseases and pests in the United States.) No difference in disease or pest susceptibility was observed in any of the transgenic sugar beet trials vs. commercial (nontransgenic) sugar beet plots. Field observation was qualitative (visual), not quantitiative. Fields were observed on a regular basis during the growing season. See Appendix 1, Field Trial Termination Reports, for more information.

C. Compositional Analysis

Data from Event T120-7 sugar beet, as well as nontransgenic counterpart beet, were analyzed statistically for nutrient content and compositional profile. Data from sugar beet raw agricultural commodities roots and tops, as well as processed sugar beet fractions were analyzed for statistical significance in comparison with literature values.

Compositional data from sugar beet roots and tops grown in three primary sugar beet-growing regions of the United States, the Red River Valley (RRV), southern Idaho, and The Imperial Valley in California were analyzed for statistical significance. Transgenic and nontransgenic samples were compared with respect to their moisture, caloric, lipid, protein, ash, carbohydrate, fiber and mineral content. The results of these analyses showed that the nutrient profiles of the sugar beet fractions from both the transgenic and nontransgenic samples were generally typical of values reported in literature (Ensminger, et. al., 1990).

Nutrient data from three processed fractions (refined sugar, molasses, dried pulp) and the roots of sugar beets grown in Fresno, California, were analyzed. Transgenic and nontransgenic samples were compared with respect to their moisture, lipid, protein, ash, carbohydrate, fiber, mineral, fatty acids and amino-acids content, as well as their sugar profiles. The results of these analyses showed that the nutrient profiles of the processed fractions from both the transgenic and nontransgenic refined sugar and molasses samples were generally similar to values reported by the USDA (1996) for granulated sugar and molasses available for human consumption. Similarly, the nutrient profiles of the sugar beet roots, and the molasses and dried pulp fractions (typically animal feed items) were similar to the standard levels reported in literature (Ensminger et al, 1990).

VI. Potential for Environmental Impact from Noncontained Use of Glufosinate-Ammonium Tolerant Sugar beet Event T120-7

A. The Herbicide Glufosinate-Ammonium and Current Uses

Ammonium-DL-homoalanin-4-yl-(methyl) phosphinate (glufosinate-ammonium) is a non-selective, non-systemic herbicide that controls a broad spectrum of annual and perennial grass and broadleaf weeds. The L-form is the active component of glufosinate-ammonium. This form is also the active portion of the naturally occurring anitbiotic bialaphos. It has a similar structure and shape as glutamic acid, and is therefore called a glutamic acid analog. It inhibits the enzyme glutamine synthetase which converts glutamic acid and ammonia into glutamine (Leason et al., 1982). L-phosphinothricin's ability to bind irreversibly with glutamic acid results in the buildup of ammonia that inhibits photophosphorylation in photosynthesis (Weld and Wendler, 1990). Phytotoxic symptoms (chlorosis and wilting) occur rapidly, especially under warm air temperature, high humidity, and bright sunshine conditions. Symptoms usually appear within 48 hours after application. Necrosis occurs in 4-7 days after application.

Glufosinate-ammonium must be absorbed through the leaves to be effective. It has limited stem uptake, and translocation within the plant is dependent upon the application rate, plant species, and stage of plant growth. Glufosinate-ammonium is rapidly degraded in the soil by microorganisms, not only in well aerated soils, but also in soils with stagnant moisture (Anonymous, 1991). Therefore, glufosinate-ammonium has very low residual soil activity and does not injure seedlings before emergence. Both glufosinate-ammonium itself and its degradation products are adsorbed to clay particles and humus materials in the soil. This greatly restricts the mobility of these compounds, despite their ready solubility in water, and prevents leaching to deeper soil layers. When used correctly, glufosinate-ammonium does not affect soil microflora or alter the number or mass of earthworm populations. Application of glufosinate-ammonium at recommended field application rates presents no hazard to fish or aquatic invertebrates. It is not a contact poison for honeybees. If used in accordance with directions for use, glufosinate-ammonium is not a hazard to birds or mammals. There were no changes in tumor incidence after lifetime exposure to glufosinate-ammonium in rats and mice. No mutagenic activity was detected in a battery of mutagenicity tests. When administered to pregnant animals, glufosinate-ammonium produced no adverse effects on fetuses at doses which were not toxic to the mothers (Anonymous, 1993).

B. Effects on Agricultural and Cultivation Practices of Sugar beet

1. Current Practices

Sugar beet (*Beta vulgaris*) is primarily grown as a spring (March-May) planted crop, however in the Imperial and Central Valleys of California sugar beets are planted at various times of the year. Sugar beet roots are typically harvested 5-6 months after planting and processed into granulated sugar and other by products. In order for sugar beet seed to be produced, the plant must remain in the soil through the winter months so that photothermal induction (a cold period) can occur. These plants will continue growth the next spring and develop a seed head that can be harvested in the fall. Most seed production occurs in areas with moderate winter temperatures (Oregon).

Sugar beet fits very well into a rotation with other crops such as small grains, corn, alfalfa and potatoes. To avoid potential disease build-up sugar beets should not be planted immediately following a previous sugar beet or legume crop. It is recommended that sugar beets be planted only once in any three to four year rotation cycle.

Rotating with sugar beet enables growers to break disease and insect cycles that become a problem in many continuous cropping situations. Sugar beet is resistant to many of the diseases and insect pests that can affect yield in small grains. The addition of sugar beet in a cropping rotation will also help eliminate certain weed problems associated with continuous cereal grain production. Grass type weeds can be substantially be reduced when sugar beet is grown in rotation with cereal grains. Volunteer sugar beet is typically not a problem in rotational crops since seed is not produced in the first year of growth and the roots are removed from the ground and processed immediately after harvest.

In sufficient numbers, weeds can significantly reduce sugar beet yields, quality and ease of harvest. Both preemergence and postemergence herbicides are available for use in sugar beet production in the United States. The preemergence herbicides can provide control of both grass and broadleaf weeds, however, crop injury can be concern and poor weed control is a possibility due to unfavorable environmental conditions or high organic matter, fine textured soils. Generally, a postemergence herbicide is used in conjunction with preemergence herbicides to get acceptable weed control.

For postemergent herbicides to be effective, timely applications to very small weeds are required. Usually 2 to 4 applications of the broadleaf herbicides are typically required for acceptable control. Grass weeds are controlled with applications of herbicides in the acetyl Co-A carboxylase family. This family of herbicide has been used extensively in the production of small grains to control grass weeds such as wild oat and foxtail. Over use of these products has lead to the development of resistance in both wild oat and foxtail in many of the sugar beet growing areas.

Therefore, control of resistant populations of wild oat and foxtail is becoming a more wide spread problem.

Most diseases are kept under control with proper crop rotation and/or planting of disease resistant sugar beet hybrids. Seedling diseases and Cercospora Leaf Spot can be minimized with the use of fungicide seed treatments or foliar sprays, respectively. Nematodes and the sugar beet root maggot (*Tetanops myopaeformis*) are the most severe insect pests in the production of sugar beets. Nematodes can only be prevented by long rotations with immune crops such as alfalfa, beans, potatoes, small grains and corn. Damage from the sugar beet root maggot can be reduced by applying a suitable soil applied insecticide.

Sugar beet roots are mechanically lifted from the ground and trucked to a processing facility after maximum sugar content is achieved. The remaining sugar beet foliage and root parts are worked into the soil with a disc or field cultivator prior to planting with following crops. Weed control must begin no later than the 4-6 leaf stage of growth of sugar beets. Current weed management practices favor the use of postemergence herbicides to achieve the greatest weed control. Soil applied products have also played an imported role in weed control in the past, but their use in sugar beets has been in decline for several years. New technologies will aid in the identification and selection of weed control systems which will reduce the application rate of herbicides (Scott and Wilcockson, 1976; Cooke and Scott, 1993).

Weed control should be continued until the 10-12 leaf stage of growth of the beets. In the critical six week period from the 4-6 to 10-12 leaf stage, for every day that weeds are allowed to compete final yield is reduced by 120-150 kg/ha (15%) per day. This loss would amount to 5,040 - 6,300 kg (about a 60% yield loss). Where weeds are never controlled and consist of tall growing species such as *Chenopoduim album* (lambsquarters), yield loss can be as great as 95%. Where shorter growing, less vigorous weeds predominate 50% yield loss is common (Scott and Wilcockson, 1976).

Kochia scoperia (Kochia), if allowed to compete with sugar beets all season can also reduce yields by as much as 95%. This weed must be controlled for the first 3 to 4 weeks of sugar beet seedling growth (Weatherspoon and Schweizer, 1969). *Chenopodium album* (lambsquarters) populations can reduce sugar beet yields by as much as 50%. Sucrose yields can also be reduced by as much as 50% (Dawson, 1946). The minimum number of lambsquarters plants per 30 meters of row which caused yield losses was 6 plants in 1980 and 4 plants in 1981 studies in Colorado (Schweizer, 1983). Similar losses by Schweizer were observed when equal mixtures of *Chenopoduim*, *Ameranthus*, and *Kochia* were present in 30 meter rows of sugar beets. By harvest, where herbicide systems had been employed, broadleaf weeks averaged 75 to 85% less than those broadleaf weeds in untreated plots (Schweizer, 1981). In the state of Washington, where beets are irrigated full season, weed control resulted if weeds were hand pulled during the first 10-12 weeks after planting. Following this period, newly germinated weeds offer little competition except when weeds overtop sugar beets. In this case competition will occur because light is a key factor in sugar beet growth.

Sugar beets exhibit marginal tolerance to registered herbicides (Cooke and Scott, 1993). Postemergence herbicides can have damaging effects if applied early in the day versus evening applications. High soil moisture levels can also increase crop damage by products such as phemmedipham and desmedipham (Bethlenfalvay and Norris, 1977). Metamitron causes greater crop injury when applied under wet soil conditions and high relative humidity (Preston and Biscoe, 1982). In the U. S., sugar beet herbicides applied to the soil range in use rate between 0.50 - 0.75 pounds active ingredient per acre (trifluralin) to 4.0 - 6.0 pounds active ingredient per acre (pebalate). An estimated average use rate per acre is between 2.5 - 3.0 pounds for the soil applied products. Postemergence herbicides are applied in the range of 0.20 pound active ingredient per acre (clopyralid) to 3.7 pounds active ingredient per acre (pyrazon). An estimated average use rate per acre would be between 1.0 - 1.5 pounds (Weed Control Manual, 1996).

2. Possible Effect of Glufosinate-Ammonium Tolerant Sugar Beet on Current Practices

Glufosinate-ammonium, a non-selective herbicide, will provide control of most annual grass and broadleaf weeds in glufosinate-ammonium resistant sugar beet including acetyl Co-A carboxylase resistant wild oat and foxtail. Glufosinate-ammonium will control larger broadleaf weeds than currently available herbicides, thus allowing more application flexibility when environmental conditions prevent the timely application required by today's herbicides. In addition, glufosinate-ammonium will provide a different herbicide mode of action in the growers' crop rotation, which is important in preventing the build up of herbicide resistant weeds. Glufosinate-ammonium is applied like any other postemergent herbicide used in any other crop. Glufosinate-ammonium tolerant sugar beet could alter current sugar beet cultivation practices in that it could allow for reduced herbicide use than currently is practiced in order to achieve the same crop yield.

3. Likelihood of Appearance of Glufosinate-Ammonium Tolerant Weeds

This topic has been thoroughly addressed in sections II.C. and II.D., and in Appendix VI., with the conclusion that in the absence of herbicide treatment, viable offspring produced from gene pollen flow from glufosinate-ammonium tolerant sugar beet to weedy relatives would have no fitness enhancement over current populations of wild or weed beets which occur naturally in nonagricultural environments.

C. Effects of Glufosinate-Ammonium Tolerant Sugar beet on Non-target Organisms

No adverse effects to beneficial organisms (earthworms, lady bugs) were noted in any trial. Populations of beneficial insects were comparable to those found in commercial sugar beet fields. (See Appendix I, for Field Trial Termination Reports.)

D. Weediness Potential of Glufosinate-Ammonium Tolerant Sugar beet

The potential of glufosinate-ammonium tolerant sugar beet to become a weed is no greater than the potential of nontransgenic sugar beet to become a weed. No differences in weediness characteristics have been observed in four (4) years of field testing between event T120-7 sugar beet and traditional nontransgenic sugar beet varieties. See Appendix I, Field Termination Reports, 1994-1997, for more detail. The potential of sugar beets to be come weeds has been discussed in great detail in section II.D., pages 17-19, of this document.

E. Indirect Effects of Glufosinate-Ammonium Tolerant Sugar beet on Other Agricultural Products

Sugar beet is grown for the root, from which sucrose is extracted and processed into refined sugar used for human consumption. By-products of sugar beet root processing are wet pulp and molasses. The pulp is dried and pelleted. Both pulp and molasses are fed to livestock. In some areas of the western region sugar beet tops (< 1%) are left in the fields following harvest for livestock (cattle, sheep) grazing, however, this amount is negligible (reference Appendix III.). Molasses and pulp can compose as much as 30% of livestock (beef cattle, 30%; dairy cattle, 30%) diet as reported in the most recent update of the EPA's Table II: Raw Agricultural and Processed Commodities and Feedstuffs derived from Field Crops, September 1995 (reference Appendix IV.). (Although tops are also reported as a feedstuff up to 20% of livestock diet, this has been disputed by the American Sugar Beet Growers Association. (Reference Appendix III.))

Refined sugar contains no protein. Thus, refined sugar derived from glufosinate-ammonium tolerant sugar beet is no different than nontransgenic sugar beet with regard to refined sugar content. Sucrose yield of glufosinate-ammonium tolerant sugar beet is comparable to that of current commercial sugar beet varieties. By-products molasses and pulp, which are used as livestock feed contain protein. Data generated by AgrEvo show that while the PAT and APH (3') II proteins are present in these commodities, they are inactive (Shilito, 1997). Furthermore, studies conducted previously on pure, active PAT protein (which is present in sugar beet tops) demonstrate that the protein is both heat and acid labile, and, that it is destroyed in the gastric juices of livestock (Schultz et al., 1997). Data also indicate that *pat* DNA, should there be any present in the by-product matricies, is also very rapidly degraded in livestock gastric juices.

F. Potential for Gene Transfer to Other Organisms

The potential for transfer of genomic information from glufosinate-ammonium tolerant sugar beet to wild species, cultivated sugar beet, and to organisms with which it cannot interbreed, and the consequences thereof has been comprehensively addressed in sections II.D., and II.E., of this document, and in Appendicies II and VI. The potential for gene transfer to other organisms can be summarized as follows: Due to the biennial nature of sugar beet, as well as the fact that populations of wild and weed beets in the U.S. is well documented and monitored (Panella and Lewellen, 1998), the risk of gene transfer via pollen from transgenic sugar beets to other organisms is negligible. Ecological fitness studies have not demonstrated that transgenic crops engineered to express herbicide tolerance exhibit increased weediness potential compared to their nontransgenic parental lines (Purrington and Bergelson, 1996).

Movement of transgenes from genetically engineered plants to microorganisms has been suggested as a risk if such plants are released into the environment. As initially stated in the USDA's Interpretive Ruling on Calgene, Inc. Petition for Determination of Regulatory Status of FLAVR SAVR™ Tomato (USDA-APHIS, 1992b), and subsequently repeated in other USDA Determination documents, "There is no published evidence for the existence of any mechanism, other than sexual crossing" by which genes can be transferred from a plant to other organisms. As summarized in these Determination documents, evidence suggests that, based on limited DNA homologies, transfer from plants to microorganisms may have occurred in evolutionary time over many millennia. Even if such transfer were to take place, transfer of the *pat* gene to a microbe would not pose a plant pest risk. Genes encoding both PAT enzymes and acetyl transferases are found in microbes in nature.

VII. Statement of Grounds Unfavorable

No unfavorable information and data have been demonstrated for glufosinate-ammonium tolerant sugar beet transformation event T120-7.

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IX. Appendicies

Appendix I.: U.S. Field Trial Reports: 1994, 1995, 1996, 1997

Appendix II.: Letter from J.R. Stander, Betaseed, Inc., 1996, to USDA/APHIS, providing ra tionale for sugar beet to be a crop considered under notification

Appendix III.: A Statement from the American Sugar Beet Growers Association discussing the fate of sugar beet tops following harvest of sugar beet

Appendix IV.: EPA's Table II: Raw Agricultural and Processed Commodities and Feedstuffs derived from Field Crops

Appendix V.: Significant Diseases and Pests of Sugar beet in the United States

Appendix VI.: A letter from Lee Panella, USDA/ARS, Fort Collins, CO, and Robert Lewellen, USDA/ARS, Salinas, CA, which addresses the issue of gene flow from cultivated sugar beet to wild, or weed species with which it has potential to outcross, and specifically addresses the issue of herbicide tolerant sugar beet with respect to its potential for increased risk of weediness respective of nontransgenic sugar beet.

Appendix VII.: Test kit instructions, including methodologies, on how to conduct PAT and APH(3')II ELISAs, respectively.....

Appendix I.: U.S. Field Trial Reports: 1994, 1995, 1996, 1997

U.S. Field Termination Report, 1994

In 1994 AgrEvo field tested Event T120-7 sugar beets at three locations, one site was in Fargo, North Dakota, one site was in Fresno, California and one site was and the other site was in Wonder Lake, Illinois. At each location transgenic sugar beets were compared to the nontransgenic counterpart. All nontransgenic plants were treated identically with herbicides which are commonly used for weed control in sugar beet production. The transformed sugar beets were sprayed with glufosinate-ammonium (2 x3 litres/hectare).

Results: No differences were observed and recorded regarding insect susceptibility and disease resistance in the transformed sugar beet versus its non-transformed counterpart. Observations in the transgenic sugar beet trials indicate insect pest and disease resistance was similar to that of nontransgenic sugar beet growing surrounding commercial fields.

No differences in the transgenic sugar beet vs. common commercial sugar beet varieties grown in the respective region were observed in plant emergence, seedling vigor, and stand establishment.

The destruction of plants at each site was carried out by cultivation or a combination of mowing and cultivation. Cultivation consisted of either disking or rototilling. Monitoring for volunteer plants was conducted the following spring (1995). No volunteers were observed.

U.S. Field Termination Report, 1995

In 1995 T120-7 sugar beet was field tested at six sites in the primary sugar beet growing regions of the USA. The sites were located in: Fresno, California; Jerome, Idaho; Twin Falls, Idaho; Fargo, North Dakota; Grand Forks, North Dakota; and Crookston, Minnesota. The objectives of the various trials were to evaluate sugar beet tolerance , weed control and glufosinate-ammonium residues in sugar beet. The transformed sugar beets were sprayed with glufosinate-ammonium At rates ranging from 200 to 600 g/ha.

Results: No significant differences in measured agronomic characteristics were observed between the transgenic and nontransgenic material. In addition, no differences were recorded in bolting characteristics of the transformed vs. nontransformed beets. Also no differences were observed in disease susceptibility of transformed vs. nontransformed beets. No negative effects resulting from treatment of the transgenic sugar beets with glufosinate-ammonium were measured. Crop tolerance was excellent and consistent weed control was achieved with glufosinate-ammonium applied two to three times at 300 g/ha.

Summary Report to the Field Release of Transgenic Sugar Beet Expressing Resistance to the Herbicide Glufosinate-Ammonium, 1996

Date of Report: August 1, 1997

Permit Number: 96-052-02r

Applicant: Vickie Forster, Registration Specialist
AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808

Dates of Release: April-June, 1996

Dates of Termination: July-October, 1996

Sites of Release (States/Number per State): Michigan/3, Iowa/1, Idaho/2, Minnesota/3, North Dakota/3, Nebraska/1, Wisconsin/1, Ohio/3, Colorado/2, California/1

Purpose of Release
To evaluate weed control and crop tolerance with glufosinate-ammonium herbicide applied to sugarbeet (*Beta vulgaris*) containing the *pat* gene which confers resistance to the herbicide glufosinate-ammonium. Several of the sites were utilized to collect samples for analysis of residues from application of glufosinate-ammonium.

Results
Glufosinate-ammonium herbicide applied 2 to 3 times at use rates of 300 to 400 g/ha provided complete control of all weeds through out the growing season. Crop tolerance was excellent.

Observations
The frequency of observations differed with each location. Each location was visited 3 or more times during the duration of the release. The area planted to transgenic sugarbeet was less than 0.5 acres per site. The transgenic sugarbeet plant population ranged from 4- 8 plants per square foot.

Herbicide Tolerance: Crop tolerance was very good up to the maximum use rates (600 g/ha) used in these experiments. One trial in Nebraska show some slight yellowing on the leaf margins at the later applications. These symptoms disappeared within two weeks after application. Transgenic sugarbeet plants were tolerant to other herbicides currently registered in sugarbeets. Transgenic sugarbeet plants were killed by an application of RoundUp or Harmony Extra. RoundUp is commonly used for chemical fallow applications and Harmony Extra is used to control weeds in cereal grains, which is a common rotational crop following sugarbeets.

Insect Susceptibility: No differences were observed between the transgenic sugarbeet trials and near by commercial fields. Beneficial insects (ladybugs and earthworms) were noted in some trials.

Disease Susceptibility: Disease resistance in transformed sugarbeet is not different from its non-transformed counterpart. Observations in the transgenic sugarbeet trials indicate disease tolerance was similar to surrounding commercial fields.

Weather Related Conditions: Most trial locations experienced near normal growing conditions through out the growing season. Stand establishment was a problem at two locations due to dry soil conditions at planting and 2-3 weeks following planting. These trials were sprayed and evaluated, however the plant stand was poor.

Physical Characteristics: Transgenic sugarbeet plants were observed from emergence through maturity. No differences were observed from typical commercial sugarbeets grown in the general area in plant emergence, seedling vigor, and stand establishment, except the locations mentioned above with dry soil conditions.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and non-transgenic plants.

Means of Plant Destruction

The destruction of plants at each site was carried out by cultivation or a combination of mowing and cultivation. Cultivation consisted of either disking or rototilling.

Time and Methods of Monitoring for Volunteers

Sites were visited one or more times in the spring of 1997 when soil temperatures reached a level at which sugarbeet emergence would be expected. No volunteer sugarbeet plants were observed to date.

Number of Volunteers Observed and Action Taken

If volunteer sugarbeet plants are observed, counts will be taken and all volunteer sugarbeet plants will be destroyed by mechanical means, removed by hand or destroyed with herbicides other than glufosinate-ammonium.

Summary Report to the Field Release of Transgenic Sugar Beet Expressing Resistance to the Herbicide Glufosinate-Ammonium, 1997

Date of Report: November 24, 1997

Permit Number: 97-029-01r

Applicant: Vickie Forster, Registration Specialist
AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808

Dates of Release: April-June, 1997

Dates of Termination: July-October, 1997

Sites of Release (States/Number per State): Michigan/4, Idaho/9, Minnesota/12, North Dakota/5, Nebraska/2, Wisconsin/1, Colorado/1, California/4, Montana/4, Wyoming/3

Purpose of Release
To evaluate weed control and crop tolerance with glufosinate-ammonium herbicide applied to sugarbeet (*Beta vulgaris*) containing the *pat* gene which confers resistance to the herbicide glufosinate-ammonium. Several of the sites were utilized to collect samples for analysis of residues from application of glufosinate-ammonium.

Results
Glufosinate-ammonium herbicide applied 2 to 3 times at use rates of 300 to 400 g/ha provided complete control of all weeds through out the growing season. Crop tolerance was excellent.

Observations
The frequency of observations differed with each location. Each location was visited 3 or more times during the duration of the release. The area planted to transgenic sugarbeet was less than 0.5 acres per site. The transgenic sugarbeet plant population ranged from 4- 8 plants per square foot.

Herbicide Tolerance: Crop tolerance was very good up to the maximum use rates (600 g/ha) used in these experiments. Transgenic sugar beet plants were tolerant to other herbicides currently registered for weed control in sugar beets. Transgenic sugar beet plants were killed by an application of non-selective herbicides RoundUp® or Harmony® Extra. RoundUp® is commonly used for chemical fallow applications and Harmony ®Extra is used to control weeds in cereal grains, which is a common rotational crop following sugar beets.

Insect Susceptibility: No differences were observed between the transgenic sugar beet trials and near by commercial fields. Beneficial insects (ladybugs and earthworms) were noted in some trials.

Disease Susceptibility: Disease resistance in transformed sugarbeet is not different from its non-transformed counterpart. Observations in the transgenic sugarbeet trials indicate disease tolerance was similar to surrounding commercial fields.

Weather Related Conditions: Most trial locations experienced near normal growing conditions through out the growing season. Stand establishment was a problem at two locations due to dry soil conditions at planting and 2-3 weeks following planting. These trials were sprayed and evaluated, however the plant stand was poor.

Physical Characteristics: Transgenic sugarbeet plants were observed from emergence through maturity. No differences were observed from typical commercial sugarbeets grown in the general area in plant emergence, seedling vigor, and stand establishment. One exception: at most locations, seed head production (bolting) is occurring in up to 14% of the plant population. If bolting is observed, the seed stalk is cut off prior to pollination and placed on the ground or the entire plant is removed from the ground and laid on the soil surface. Trial locations are visited weekly to check for the development of seed stalks.

Weediness Characteristics: Weed populations have been normal in the sugarbeet growing areas. Weed control with glufosinate ammonia (glufosinate-ammonium) has been good to excellent. The glufosinate-ammonium resistant sugar beet has shown no injury from applications of glufosinate-ammonium, however, approximately 5-30% of the sugar beet plants have been killed by the applications of glufosinate-ammonium due to the occurrence of non-transgenic types in the various seed lots.

Means of Plant Destruction

The destruction of plants at each site was carried out by cultivation or a combination of mowing and cultivation. Cultivation consisted of either disking or rototilling.

Time and Methods of Monitoring for Volunteers

Sites will be visited one or more times in the spring of 1998 when soil temperatures reach a level at which sugar beet emergence would be expected.

Number of Volunteers (1996 Trials) Observed and Action Taken

The trial locations from last year continue to be monitored and any volunteer plants are controlled with tillage or herbicide applications.

Appendix II.: **Letter from J.R. Stander, Betaseed, Inc., 1996, to USDA/APHIS, providing rationale for sugar beet to be a crop considered under notification.**

BETASEED

Betaseed, Inc 3452 East 3700 North P O Box 858 Kimberly, ID 83341 Telephone (208) 423-4648 Fax (208) 423 4779

March 28, 1995

Dr. John H. Payne, Acting Director
Biotechnology, Biologics, and Environmental Protection
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Dear Dr. Payne:

Thank you for your letter of March 15, 1995 regarding the possibility of adding sugarbeets to the list of crops eligible for notification.

I would like to formally petition APHIS to consider sugarbeets for notification. I have attempted to demonstrate that sugarbeets can be field tested in accordance to the performance standards as listed in §340.3(c). I have also enclosed pertinent literature.

If I can be of assistance in the clarification of any statements or in providing additional information, please don't hesitate to contact me. I would also recommend Dr. Robert T. Lewellen as a noted expert in the field of sugarbeet genetics and production. Dr. Lewellen is a USDA ARS Research Geneticist located at Salinas, CA (phone: 408-755-2833).

Sincerely yours,
BETASEED, INC.

J. R. Stander
Manager of Research

Enclosures

1. Description of the sugarbeet plant

Sugarbeet, *Beta vulgaris* L., is a member of the family *Chenopodiaceae*. It is a cross-pollinating biennial plant that is grown commercially as an annual root crop. The natural distribution of wild species of *Beta* (*B. vulgaris ssp. maritima*, ssp. *macrocarpa*, ssp. *adanensis*) is found largely along the Atlantic coasts of Europe and North Africa and in the Mediterranean (*maritima*),while ssp. *macrocarpa* predominates in the western Mediterranean basin and ssp. *adanensis* is centered in the eastern Mediterranean area (*Smartt* 1992). The ssp. *patula* is confined to a small desert island near Madeira (*Letschert* 1992).

The sugarbeet is a biennial plant which develops a fleshy taproot and a stunted stem with a leaf rosette in its first year of vegetative growth. The majority of the wild Mediterranean *Vulgares* beets (*Beta maritima, B. macrocarpa, B. patula*), however, are annuals. Cultivated sugarbeets require a period of low temperature (thermal induction) to switch from vegetative to reproductive growth. The length of thermal induction required is determined genetically, and if short enough, seed stalk development may be induced by low spring temperatures already in the first year. Sugarbeets are killed by frost and temperatures below -5 °C.

In the beginning of the reproductive stage the flower stalk elongates (bolting) and forms an inflorescence consisting of multiple flowers located in the terminal portions of the main axes and on the lateral branches. The sugarbeet has a flower that commonly consists of a tricarpellate pistil surrounded by five anthers and a perianth of five narrow sepals. Petals are absent. Each flower is subtended by a slender green bract.

The terms monogerm and multigerm are commonly used to describe sugarbeet seed. However, botanically speaking, they are fruits. 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; therefore, a multiple fruit is formed by the aggregation of two or more flowers. The multigerm beet seed is formed by aggregation of two or more enclosed fruits. A monogerm seed is formed when a flower occurs singly. Flowers generally reach anthesis about 5 to 6 weeks after the initiation of reproductive development.

After dehiscence of the mature anthers the globular pollen is transmitted by wind and occasionally by insects. Sugarbeet pollen is extremely sensitive to moisture. But even under dry conditions its viability is lost within 24 hours.

Sugarbeet is normally strongly self-sterile setting few or no seeds at all under strict isolation. The underlying genetic mechanism could be explained by two series of multiple sterility alleles (S_1 - S_n, Z_1 - Z_n). The setting of some seeds after selfing, so called pseudo-compatibility, is due to break-down of the incompatibility-mechanism. It is more or less pronounced in different genotypes and is very highly influenced by environmental conditions, especially temperature.

Most characters for which sugarbeet breeders select are expressed during vegetative growth

(characteristics such as root yield, sugar content, impurity levels, resistance to bolting, resistance to diseases, etc.).

2. Wild relatives of sugarbeets in the U.S.

Some relatively small wild populations of *Beta* have become established in California due to the mild climate. These populations are described as *B maritima, B macrocarpa,* and *B vulgaris*. The populations of *B maritima* and *B macrocarpa* are suggested to have developed from seed contaminants or from seed intentionally imported into California. The population of *B vulgaris* is believed to have developed from sugarbeet itself. No wild populations of *Beta* have been reported in the U.S. outside of California.

In 1928 *Carsner* reported wild beet populations in most of the older beet growing areas of California (see: *McFarlane*). These beets were found in Imperial, Santa Clara, Ventura, San Bernardino, Los Angeles, and Orange Counties. He speculated that these beets were either *B maritima* or hybrids between *B maritima* and sugarbeet. He was of the opinion that wild beets had been introduced into California as seed contaminants. With the exception of the wild populations in Imperial and Santa Clara Counties there have been no further reports of these populations.

McFarlane (1975) identified the wild beets in Imperial County as *B macrocarpa* rather than *B maritima. B macrocarpa* is a species that occurs naturally in the Canary Islands and along the Mediterranean coastline. He speculated that seed of *B macrocarpa* were imported as contaminants in seed or in feed grain. He reported the existence of numerous naturally occurring hybrids between plants of *B macrocarpa* and commercial sugarbeets in the Imperial Valley. *Abe* (1988) and *Lewellen* (personal communication), however, report that sugarbeet and *B macrocarpa* do not readily produce viable hybrids.

Dahlberg and *Brewbaker* (1948) referred to the population of *B maritima* in Santa Clara County as the "Milpitas wild beet". They speculate that this population 1) established itself from *B maritima* which was inadvertently imported from Europe along with sugarbeet seed for the fledgling sugarbeet industry, or 2) may have become established from seed brought in by the Franciscan Fathers when they established the Santa Clara and other missions in the late 1700's. The area where these beets were found is now highly urbanized and is no longer an area where sugarbeets are commercially grown (*Lewellen* personal communication).

Johnson and *Burtch* (1959) reported the development of weed beets in California. They describe sugarbeets which evolved into annual plants and became a weed problem. Recent surveys would indicate that such populations are restricted in size and appear to be localized in the Gilroy/Hollister area (*Lewellen* personal communication).

In summary it can be said that most of reports of wild beet populations in California were old reports with little or no follow-up study. With the exception of the wild population of *B macrocarpa* in Imperial Country and the wild population of *B maritima* in Santa Clara County which are known to exist, the other populations may have been eliminated (*Lewellen* personal communication). These

known populations have been established for many years and are small populations which have shown little propensity to spread. The Imperial Valley is the only one of the areas where wild beets have been reported where commercial sugarbeets are currently being grown.

The known persistent population of weed beets which evolved from sugarbeets is small and not of great concern.

3. Risk of gene transfer - performance standards

a. Root trials

Bolters do not normally occur in sugarbeet yield trials or in disease evaluation trials, and precocious bolters can be removed. Therefore the risk of gene transfer from such trials with transgenic plants is basically non-existent.

Although uncommon, in some conditions it is possible for "groundkeepers" to become established and bolt the following year. Groundkeepers are volunteer plants which develop from meristematic tissue. Such plants which might survive normal tillage/cropping practices are obvious with routine monitoring and easily removed by hand.

b. Seed increases

i. Risk of gene transfer via interpollination with wild beets

Wild beet populations in the U.S. are rare and are only known to occur in California. There is therefore no risk of gene transfer via pollen to wild beet populations in states other than California. In California because the populations of *B maritima* and weed beets developed from *B vulgaris* are small and isolated, the risk of gene transfer is minimal requiring only the obvious precautions for isolation.

ii. Risk of gene transfer via interpollination with other sugarbeets

Bolting beets normally occur only in fields where beets are grown specifically for the production of seed.

(1) Willamette Valley

Beet seed production is generally highly specialized and localized. All commercial sugarbeet produced in the U.S. is grown in the Willamette Valley of Oregon which is itself geographically distant from the sugarbeet production areas. In addition to commercial seed much of the stock seed is also produced there. Most of the seed is produced by a single seed cooperative. Other companies which produce seed independently work together with the cooperatative to minimize problems with isolation. Because of the degree of control which can be exercised by an individual company the maintenance of desired isolation and potential gene transfer, is relatively easy to control.

(2) Other areas

In addition to seed which is produced in the Willamette Valley, there are smaller seed isolations established by breeders on or adjacent to individual breeding stations. Because these breeding stations are geographically distant from the seed production areas, and usually distant from other breeding stations, isolation and potential gene transfer are essentially under the control of the individual breeder.

Breeding stations are generally in or near major commercial sugarbeet production areas. Bolting beets are normally rare in commercial sugarbeet fields. Precocious bolters which might pose a gene transfer risk can be easily removed by hand.

The exception to the stated generalization regarding the rarity of bolting beets excepting for seed productions is in certain California commercial beet productions which are by design overwintered prior to harvest.

iii. Certification distances

The Oregon seed certification isolation distance between sugarbeets with different pollen sources is 3200 feet. The certification distance between sugarbeets and pollinators of other *Beta* species (i.e. fodder beet, red beet, swiss chard) is 8000 feet.

iv. Volunteers

It is rare for sugarbeet volunteers to persist in the environment. An exception is noted above in the discussion relative to the development of a small and isolated weed beet population in California. Even casual monitoring would have prevented the occurrence of that isolated event.

c. Summary

Because of the biennial nature of sugarbeets the risk of gene transfer from transgenic sugarbeets involved in field trials is remote. Because bolting beets are uncommon excepting in fields or plots grown specifically for seed production, there is a high degree of control which can be exerted by the breeder over isolation distances. Thus if proper performance standards are exercised the risk of gene transfer via pollen from transgenic sugarbeets is negligible.

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Appendix III.: **A Statement from the American Sugar Beet Growers Association dis cussing the fate of sugar beet tops following harvest of sugar beet.**

American Sugarbeet Growers Association
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Washington, DC 20005
Telephone: 202/833-2398
FAX: 202/833-2962

Luther A. Markwart
Executive Vice President

December 29, 1996

Ms. Vickie Forster
Registration Specialist
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Little Falls Centre One
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Dear Ms. Forster:

In response to your request about the use of sugarbeet tops in the U.S., I am pleased to provide the following information.

> The tops of sugarbeet plants are separated from sugarbeet roots by various mechanical means at harvest. Based on information from growers in all growing regions of the U.S., more than 99% of the beet tops are left in the field and tilled into the soil for decomposition. The remaining tops are fed to cattle or sheep, mostly by grazing the sugarbeet fields after harvest. Infrequently, the tops are hauled from the field to be fed to livestock. However, we do not know of any growing region where sugarbeet tops are transported across state lines for use as a feed commodity.

The American Sugarbeet Growers Association (ASGA) has as its members regional sugarbeet growers associations, and ASGA works closely with the regional associations from all sugarbeet growing regions. The regional associations have as their members sugarbeet growers in their respective regions, and most sugarbeet growers belong to a regional association.

Sincerely,

Luther Markwart
Executive Vice President

Appendix IV.: EPA's Table II: Raw Agricultural and Processed Commodities and Feedstuffs derived from Field Crops.

Table II (September 1995)

RAW AGRICULTURAL AND PROCESSED COMMODITIES AND FEEDSTUFFS DERIVED FROM FIELD CROPS

CROP	RAW AGRICULTURAL COMMODITY	PROCESSED COMMODITY	FEEDSTUFF		PERCENT OF LIVESTOCK DIET[1,2]			
				% DM[3]	BEEF CATTLE	DAIRY CATTLE	POULTRY	SWINE
[illegible]ana[9]	whole fruit							
Barley[10]	grain[11] hay straw	pearled barley flour bran	grain[11]	88	50	40	75	80
			hay	88	25	60	NU	NU
			straw	89	10	10	NU	NU
Bean[12]	bean, succulent seed							
Beet, garden	root tops (leaves)							
Beet, sugar	root tops (leaves)	sugar, refined[13] pulp, dried molasses	tops (leaves)	23	20	10	NU	NU
			pulp, dried	88	20	20	NU	NU
			molasses	75	10	10	NU	NU
Blackberry[14]	berry							

Appendix V.: Significant Diseases and Pests of Sugar beet in the United States

FUNGAL PATHOGENS

Cercospora Leaf Spot: *Cercospora beticola*

Cercospora leaf spot is one of the most important, widespread, and destructive fungal diseases affecting beets. In the U.S. the disease is most prevalent from Michigan/Ohio to Colorado and northern Texas. The disease develops rapidly when day temperature is 25° to 33°C and the humidity is high. First symptoms are small, whitish spots scattered over the surface of older leaves. The spots increase in size, becoming brownish or purplish in color. Individual spots are usually circular but several may coalesce into larger areas of dead tissue. Mature spots, about 1/4-inch in diameter, become gray as the fungus produces spores. Leaves may become yellow and die. As leaves die, the crown becomes cone-shaped with a rosette of dead leaves at the base. This disease can cause reduced tonnage and sucrose and increased impurities. Losses of 30 percent in recoverable sucrose are fairly common under moderate disease conditions. In addition roots of affected plants do not store as well after harvest as roots of healthy plants. Sugar beet hybrids range from susceptible to moderately tolerant to Cercospora. Weather conditions which are optimal for the fungus and frequent rainfall which washes fungicide from the leaves favor epidemics. Control is accomplished through resistant varieties and preventive fungicide applications. Control is confounded by the development of strains of Cercospora with tolerance to the tin fungicides.

Rhizoctonia Root Rot: *Rhizoctonia solani*

Rhizoctonia root and crown rot caused by *Rhizoctonia solani* occurs in many sugarbeet production areas and is the most common root disease in of sugarbeet in the U.S. It has been known to cause up to 50% yield loss. The causal fungus, *Rhizoctonia solani*, occurs in agricultural soils throughout the world and attacks many crop species. The fungus survives on plant debris in the soil. This disease is most common during spring and summer when conditions are warm 25° to 33°C and soils are moist. The fungus grows through the soil and infects the root and crown of plants. Rhizoctonia occurs in most soil types, but is most severe in heavy, poorly drained soils. The root and crown are partially or completely destroyed. Control measures include rotation, water management, and resistant varieties.

Fungal Seedling Diseases

Sugarbeet is susceptible to numerous seedling diseases expressed as seed decay, and preemergent or postemergent damping-off. Significant losses are known to occur from the following soilborne fungi: *Pythium ultimum, P. aphanidermatum, Rhizoctonia solani* and *Aphanomyces cochlioides.* Depending on the pathogen, most of the seed tissue is susceptible to infection, including non-germinated seed, germinating radicle, and emerging seedling up through the four- to six-leaf stage. Preemergence damping-off appears as darkened lesions on the emerging radicle and causes death of the radicle and seedling. Postemergence damping-off appears as a lesion on the seedling root or crown tissue, and causes the seedling to wilt, and possibly die. Plants that survive infection will not grow vigorously, resulting in greatly reduced yields. *Pythium ultimum* is widespread in soil and attacks many crops. It infects unprotected seedlings at temperatures favorable for

germination of beet seed (24° to 30°C), especially in winter and spring under conditions of warming soils with a high moisture content. It primarily causes a preemergence damping-off, but under moist conditions a postemergence damping-off may occur. *Pythium aphanidermatum* attacks seedlings only in warm soils (30° to 35°C) with abundant soil moisture. *Rhizoctonia solani* and *Aphanomyces* spp. are problems primarily on emerged seedlings when temperatures are above 20° to 30°C.

VIRAL PATHOGENS

Virus Yellows

Beet Yellows Virus (BYV)

Beet Western Yellows Virus (BWYV)

Beet Mosaic Virus (BMV)

The yellowing viruses of sugarbeet became a serious threat to stable sugarbeet production in many places throughout the world in the 1940s and continue to cause serious problems. In the U.S. virus yellows is primarily found in California, but may also occur in other western states. The yellowing viruses important in the U.S. are primarily Beet Yellows Virus (BYV), Beet Western Yellows Virus (BWYV), and Beet Mosaic Virus (BMV). These viruses, all of which are aphid borne, may occur alone or in combination as a viral complex. Virus yellows has been known to cause severe losses in sugarbeet both in yield and in percent sugar. Symptoms of beet yellows virus and beet western yellow virus are very similar and typically first appear on older leaves that begin to yellow in the area between the veins where small reddish-brown spots often appear, giving the leaves a distinct bronze cast. Eventually leaves become thick, leathery, and brittle. Severe strains of beet yellows virus first cause a vein etching of the heart leaves, followed by yellowing of entire leaf blades or sectors of older leaves. These diseases are vectored primarily by the green peach aphid, *Myzus persicae*, and the black bean aphid, *Aphis fabae*. The aphids obtain beet yellows and beet mosaic viruses primarily from overwintering beets; beet western yellows and beet mosaic viruses have a very wide host range, however, including plants in the crucifer and composite families. Disease potential is greatest in years when aphids are able to colonize beets early in spring and multiply rapidly; crop loss can be considerable, ranging up to 2% or more per week of infection. Plants infected at early stages of development suffer the heaviest losses.

Beet Curly Top Virus (BCTV)

Beet curly top virus is a single-stranded DNA virus in the Geminivirus group. Curly top is a highly destructive disease of sugarbeets and may occur throughout the arid and semi-arid areas of the western and southwestern U.S. The beet curly top virus may also cause significant losses in tomatoes, beans, and cucurbits. In addition to the U.S. curly top has also been found in Argentina, Brazil, Uruguay, Turkey, and Iran. Beet curly top virus has an extensive host range consisting of more than 300 species in 44 plant families. It is vectored by the beet leafhopper, *Circulifer tenellus*, which has a wide host range, a high reproductive capacity, and can migrate long distances to cultivated areas from its breeding grounds in the coastal foothills of California or the desert areas of other western states. The leafhopper overwinters on a wide range of annual and perennial weeds and readily acquires the virus when it feeds on infected plants. In spring, it mi-

grates to agricultural lands when the overwintering host plants dry out. The virus causes the leaves to become dwarfed, crinkled, and to roll upward and inward. Veins on the lower side of infected leaves are irregularly swollen. If roots are cut crosswise, dark vascular rings can be observed. Plants may be severely stunted or killed depending upon the strain of the virus, the level of genetic susceptibility, and the growth stage of the plants at the time of infection. Control is through the utilization of resistant varieties and timely planting to avoid the exposure of seedlings or young plants to the virus. Some protection can also be obtained through the application of insecticides.

Rhizomania: Beet necrotic yellow vein virus (BNYVV)

Rhizomania ("root madness" or "bearded root") is one of the most destructive diseases of sugarbeet. Rhizomania is widespread in temperate regions of Europe and Asia and also occurs in California, Texas, Idaho, Oregon, Colorado, Wyoming, Nebraska, and Minnesota. The causal agent is beet necrotic yellow vein furovirus (BNYVV), whose vector is *Polymyxa betae*, a soil-borne plasmodiophoraceous fungus. The fungus is an obligate parasite, reproducing only within living tissue of its host. Disease development is influenced by the fungus, which is enhanced by saturated soil conditions from rain, irrigation, or poor soil drainage. In infested fields, most sugarbeets are affected: roots are usually small, sugar yields are poor, and losses can be as high as 100%. Recent studies suggest that additional losses in fields with infected beets may be the result of secondary invasion by other root pathogens, such as *Phytophthora* or *Pythium*. The disease has caused losses in root yield as high as 80 % in some European fields. Losses of 20 % to 50 % in sugar yield and reduced juice purity are common in infested areas. Typical disease symptoms are root stunting and proliferation of lateral rootlets on the main taproot ("bearded roots"). Cross sections of infested taproots show darkening of vascular vessels. Leaves may have an upright posture or become flabby and may wilt from lack of water. Veinal yellowing and leaf necrosis may occur if the virus becomes systemic, this, however, is uncommon. Viruliferous resting spores (cystosori) of *Polymyxa betae* can apparently survive in uncultivated soil for as long as 15 years. Use of tolerant varieties in conjunction with planting into cool soils will reduce losses to this disease.

INSECT PESTS

Sugarbeet Root Aphid: *Pemphigus populivenae (betae)*

Pemphigus populivenae (betae), and/or closely related *Pemphigus* spp., is one of the most widespread pests of sugarbeet in the U.S. and occurs throughout the major sugarbeet growing areas of North America. Economically damaging infestations have been reported from Texas to Alberta, Canada, and from California to Michigan. Sugarbeet root aphid, as the name implies, is restricted to the roots; generally, the aphid is associated with fibrous roots rather than the main storage root. Winged aphids may occasionally be seen in woolly wax masses in the crown as they crawl up from the roots to fly to new hosts. Wingless forms found on roots are yellowish in color and secrete a dull, white waxy substance, giving the root a mealy appearance. Severely infested plants become chlorotic and wilt easily; under conditions of prolonged moisture stress, the storage root becomes flaccid and rubbery. Infestations in the field often appear as circular or elliptical patches in which the foliage on plants is wilted or, in extreme cases, collapsed and dying.

Sugarbeet root aphid infestations have nearly always been more severe under dry soil moisture conditions, due either to dry years under dryland conditions in the upper Midwest, or to the reduced use of water in the irrigated areas of the west and southwestern U.S. Genetic resistance is available and has been incorporated in some adapted varieties. Rotation and water management may also be effective in reducing damage.

Sugarbeet root maggot: *Tetanops myopaeformis*
The sugarbeet root maggot is probably the most destructive insect pest of sugarbeet in the U.S. Sugarbeet root maggots routinely cause economic losses to sugarbeet crops in the Minnesota/North Dakota and intermountain sugarbeet production areas. The maggots feed on succulent roots and cause the plants to wilt and become stunted. They may sever taproots of small plants, causing them to die. Their feeding scrapes the root surface and may also provide entry points for soilborne pathogens. The sugarbeet root maggot larva is a small, legless maggot with no distinct head or eyes. It is white in color and has two dark mouth hooks at the pointed end that are used for feeding. The adult fly is about the size of a housefly with a shiny black body with brownish spots at the base of the wings. Insecticide application reduces damage from the maggots.

PARASITIC NEMATODES
Sugarbeet Cyst: *Heterodera schachtii*
Root Knot: *Meloidogyne arenaria, M. incognita, M. javanica,* and *M. hapla*
False Root Knot: *Nacobbus aberrans,* and *N. dorsalis*
At least 29 species of nematodes within 16 genera are parasitic on sugarbeet. The loss in sugarbeet production attributed to nematodes is estimated to be 10%. A single species, *Heterodera schachtii*, the sugarbeet cyst nematode, accounts for more than 90% of that loss. Plant parasitic nematodes survive in soil and plant tissues and several species may exist in a field. They have a wide host range, vary in their environmental requirements, and in the symptoms they induce. Infestations of sugarbeet cyst nematode may be localized or spread over an entire field. In heavily-infested soils, seedling emergence may be delayed or seedlings may be killed before emergence, resulting in a reduced stand. Seedlings infested with sugarbeet cyst nematodes may be predisposed to secondary infection by soilborne fungi. This nematode is widespread in many growing areas in the Midwest and intermountain areas. *Meloidogyne incognita* and *M. javanica* are the most damaging root knot nematode species found in sugarbeet. Swellings (galls) can be seen on fibrous roots and the tap root, which may have a warty appearance Heavy infestation by root knot nematodes in sandy soils may cause plants to wilt and collapse. The false root knot nematode is known to cause severe damage to sugarbeet. Symptoms of false root knot nematode infestation are similar to those produced by *Meloidogyne* spp. Nematodes live on the thin film of water that surrounds each soil particle and are, thus, very sensitive to dry soil conditions. Nematodes move very slowly in the soil but are moved in running water or contaminated equipment. The disease and insect pests discussed above will effect glufosinate-ammonium tolerant sugar beets in the same way that they effect traditional sugar beet hybrids. Disease tolerance, in glufosinate-ammonium tolerant lines, has been evaluated and is similar to non-transformed isolines (hybrids with the same genetics as the transformed line). It is foreseen that glufosinate-ammonium tolerant sugar beets will eventually be developed with disease tolerance to fit all

sugar beet growing areas.

Appendix VI.: A letter from Lee Panella, USDA/ARS, Fort Collins, CO, and Robert Lewellen, USDA/ARS, Salinas, CA, which addresses the issue of gene flow from cultivated sugar beet to wild, or weed species with which it has potential to outcross, and specifically addresses the issue of herbicide tolerant sugar beet with respect to its potential for increased risk of weediness respective of nontransgenic sugar beet.

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January 9, 1998

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Dear Ms. Forster,

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. Then, 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 it the first year, and sometimes these will set a little seed. This 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* sp. *vulgaris*) in the United States that are sexually compatible with sugar beet.

There have been reports in the literature of sugar beet that 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 over 100 years, and, in that time, no weed beet problem has ever occurred. And we have much better varieties, with fewer bolters, than was the case even thirty years ago.

The story in California is a little bit different due to the climate and historical introduction of cultivated 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 is a wild beet population, the so-called Milpitas wild beet, in the San Francisco Bay area. It is most likely a mixture of escaped and annualized cultivated beet, introduced by the early Spanish settlers, with escaped sugar beet from the early sugar beet culture in this area (began in the last half of the 1800s). This is, however, an area in which sugar beet are no longer grown.

There is also a population of wild weed beets in the Imperial Valley of California. These are another subspecies of *Beta vulgaris*, *B. vulgaris* spp. *macrocarpa*. They are thought to have been introduced in the early settlement of Imperial Valley from the Canary Islands by Portugese immigrants. They are a weed problem in this area. Dr. Lewellen has, however, done some research on this species and it is his opinion that it does not outcross to sugar beet. The are a number of factors supporting this conclusion. First, these plants bolt and flower too early to hybridize with sugar beet - their seed has usually matured before sugar beet bolts and flowers in May to June. *Macrocarpa* is not sexually compatible in crosses with sugar beet. 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 in nature. Additionally, this population of *B. v.* spp. *macrocarpa* is totally self-fertile. Even in the greenhouse, crosses of *B. v.* spp. *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 because the flowering period of bolted sugar beet comes much later in the spring than that the flowering of *B. v.* spp. *macrocarpa*.

Dr. Lewellen feels that any of the wild beets reported in this area that are not the specific *B. v.* spp. *macrocarpa* types were derived from seed from early bolting sugar beet, and these have not been a persistent weed problem. Similarly other annual beets that are periodically reported in California are probably the result of shattered seed of easier bolting, overwintered sugar beet. These do not seem to persist in nature - the exception being the Milpitas wild beet. Some wild beets in California are thought to have arisen from imported Southern European seed, where in the European seed home sugar beet was outcrossed to wild weedy beets of *B. v.* spp. *maritima*. Again, with the exception of the Milpitas beet, these have not persisted in nature. Similar wild weedy beets are not native

occur in Oregon where the USA commercial sugar beet seed is grown.

Finally, 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.

For these reasons, I believe there is very little risk of a herbicide-resistant sugar beet hybridizing with a weed beet population. And in the remote possibility that this would happen, I do not think that there would be a potential weed problem. There are no persistent wild beet populations anywhere in the United States, outside of the one in the San Francisco Bay area, where sugar beet is no longer grown. Sugar beet can be controlled with many classes of herbicides and even a weed beet population resistant to a couple of herbicides, if it could persist under our climatic conditions, could be easily controlled before it became a serious weed problem.

Sincerely yours,

Lee Panella
Chair, Sugarbeet Crop Germplasm Committee

R. T Lewellen
Research Geneticist

Appendix VII.: Test kit instructions, including methodologies, on how to conduct PAT and APH(3')II ELISAs, respectively.....