96-068-01p

AgrEvo

A company of Hoechst and NOR-AM

March 8, 1996

Mr. Michael A. Lidsky
Deputy Director
USDA, APHIS, BBEP, BCTA
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Re: **Petition for Determination of Nonregulated Status for Glufosinate Resistant Soybean Transformation Events**

Dear Mr. Lidsky:

AgrEvo USA Company is submitting a Petition for Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS) for Glufosinate Resistant Soybean (GRS) Transformation Events **W62, W98, A2704-12, A2704-21 and A5547-35.**

This petition requests a determination from APHIS that GRS transformation events, and any progeny derived from crosses of events with traditional soybean varieties, and any progeny derived from crosses of GRS events with transgenic soybean varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340. The petition contains a full statement explaining the factual grounds why GRS transformation events should not be regulated under 7 CFR Part 340, including data and information required as set forth in paragraph (c) of 7 CFR 340.6. This petition does not contain any trade secrets or confidential business information (CBI) and is so marked.

Accompanying two copies of the petition and appendices 1-2 , is one copy of certain literature reprints. All literature cited in the petition is not included in the reprints since the excluded literature is either a book, a government document, or a manuscript that is readily available to the public at many libraries in the United States. If these reprints are required for you to consider this petition complete, please contact me by March 28, 1996.

#2 3/18

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

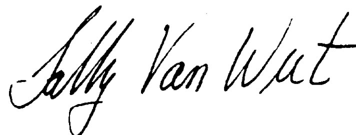
This petition contains the changes requested following your review of our initial submission (95-335-01P) in December 1995. The initial submission contained data specific to GRS event W62 and W98. The current petition adds three more events, A2704-12, A2704-21, and A5547-35. The following changes were made per BBEP's request:

1. Identification by name of the all events for which AgrEvo is requesting nonregulated status.
2. Mendelian data, Southern blots to give basic copy number estimates for the *pat* and *ampR* gene for the additional events A2704-12, A2704-21, and A5547-35, and ß-lactamase expression in these events.
3. Changing of the word 'Molecular' to 'Genetic" on page 29.
4. Clarification of positive controls mentioned on page 39 by addition of data on page 49.
5. Addition of a literature citation to justify a statement in the 2nd paragraph on page 53.
6. Replacement of page 46 in Appendix 2 with a legible page.

Two requests were not honored. AgrEvo did not provide cleaner Southern blots for Figures IV. 2. and 4. because they are not available.

Please contact me at (302) 892-3155 if you have any questions concerning our petition.

Best Regards,



Sally Van Wert, Ph.D.
Manager, Regulatory Affairs - Biotechnology

Enclosures (2)

96-068-01p

Petition for Determination of Nonregulated Status:

Glufosinate Resistant Soybean Transformation Events

The undersigned submits this petition under 7 CFR 340.6 to request that the Director, BBEP, make a determination that the article should not be regulated under 7 CFR 340.

Submitted by:

Sally Van Wert

Sally Van Wert, Ph.D.
Manager, Regulatory Affairs - Biotechnology

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Arno Schulz, Paul Umbeck

March 8, 1996

Contains No Confidential Business Information

Summary

AgrEvo USA Company is submitting a Petition for Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS) for Glufosinate Resistant Soybean (GRS) Transformation Events **W62, W98, A2704-12, A2704-21 and A5547-35**. AgrEvo requests a determination from APHIS that GRS transformation events, and any progeny derived from crosses of GRS events with traditional soybean varieties, and any progeny derived from crosses of GRS events with transgenic soybean varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340. Events W62 and W98 are considered regulated articles because they contain sequences from the plant pests *Agrobacterium tumefaciens*, alfalfa mosaic virus (AMV), and cauliflower mosaic virus (CaMV). The events A2704-12, A2704-21 and A5547-35 are considered regulated articles because they contain sequences from the plant pest CaMV and *A. tumefaciens*.

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

Transformation events W62 and W98 are soybean material of maturity groups V and III, respectively, that contain stably integrated genes which encodes phosphinothricin-N-acetyltransferase (PAT) and β-glucuronidase (GUS). The PAT enzyme catalyzes the conversion of L-phosphinothricin (PPT), the active ingredient in GA, to an inactive form, thereby conferring resistance to the herbicide. The *bar* gene in events W62 and W98 is the native gene isolated from *Streptomyces hygroscopicus*. The GUS enzyme is a scorable marker useful to plant molecular biologists in the selection of transformants. The enzyme is encoded by the *gus* gene derived from *Escherichia coli*. The events A2704-12, A2704-21 and A5547-35 are soybean material of maturity groups II and V. However, they contain only a stably integrated gene which encodes PAT. The *pat* gene in these events is a synthetic version of the 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. Plasmid DNA containing the gene(s) was coated on particles and introduced into soybean protoplasts by particle acceleration. Southern blot analyses show events W62 and W98 contain approximately 2 and 12 copies of the *bar* and *gus* genes, respectively. The

events A2704-12, A2704-21 and A5547-35 contain 4, 5 and 1 copy of the *pat* gene, respectively.

Genetically engineered GRS will provide a new weed management tool to soybean growers. GA is currently registered in the United States as a nonselective herbicide for both non-crop and crop uses. It is highly biodegradable, has no residual activity, and has very low toxicity for humans and wild fauna. GRS may positively impact current agronomic practices in soybean 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 soybean 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 needs; and 7) allowing the application of less total pounds of active ingredient than used presently.

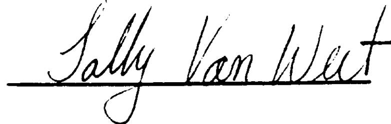
Events W62 and W98 have been field tested by AgrEvo USA Company and Asgrow Seed Company since 1990 in the primary soybean growing regions of the United States, including Puerto Rico. These tests have occurred at approximately 195 sites under field release authorizations granted by APHIS (USDA authorizations: permits 90-274-05, 91-051-03, 91-203-01, 92-043-02, 92-043-03, 93-090-01, 93-047-02, 93-047-03; notifications 93-120-31, 93-120-35, 93-127-02, 93-270-03, 94-080-03, 94-090-02, 94-131-01, 95-034-02, 95-069-01, 95-069-02, 95-069-03, 95-069-04, 95-069-05, 95-069-06, 95-069-07, 95-069-08, 95-069-09, 95-069-10, 95-069-11, 95-069-12, 95-079-02, 95-115-04, 95-135-04, 95-142-02). Events A2704-12, A2704-21 and A5547-35 were also evaluated in the field in 1995 at 2 sites under authorizations granted by APHIS (notifications: 95-034-02 and 95-122-03). Data collected from these trials, laboratory analyses, reports, and literature references presented herein demonstrate that GRS events: 1) exhibit no plant pathogenic properties; 2) are no more likely to become a weed than non-modified soybean; 3) are unlikely to increase the weediness potential of any other cultivated plant or native wild species; 4) do not cause damage to processed agricultural commodities; and 5) are unlikely to harm other organisms that are beneficial to agriculture. Transformation events W62 and/or W98 have also been field tested in Canada.

Primary transformation events W62, W98, A2704-12, A2704-21 and A5547-35 have been crossed with Asgrow's proprietary lines. The primary transformation events and their progeny are collectively referred to as GRS transformation events in this petition.

AgrEvo USA Company requests a determination from APHIS that GRS transformation events W62, W98, A2704-12, A2704-21 and A5547-35, and any progeny derived from crosses of GRS events with traditional soybean varieties, and any progeny derived from crosses of GRS events with transgenic soybean varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340.

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.



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ACRONYMS AND SCIENTIFIC TERMS

ampR - ampicillin resistance gene
AMV - alfalfa mosaic virus
bar - phosphinothricin acetyltransferase gene (origin *S. hygroscopicus*)
CaMV - cauliflower mosaic virus
ELISA - enzyme linked immunosorbent assay
GA - glufosinate-ammonium
GRS - glufosinate resistant soybean
GS - glutamine synthetase
gus- β-glucuronidase gene
GUS - β-glucuronidase
HPLC -high pressure liquid chromatography
NOS - nopaline synthase
PAT - phosphinothricin acetyltransferase
pat - phosphinothricin acetyltransferase gene (origin *S. viridochromogenes*)
PCR - polymerase chain reaction
PPT - phosphinothricin
RuBP - ribulose-1,5-bisphosphate
SSU - small subunit
TLC - thin layer chromatography

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Statement of Grounds for Nonregulated Status

I. Rationale for Development of Glufosinate Resistant Soybean

Of the eight major oilseeds traded in international markets, soybean, *Glycine max* (L.) Merr., production dominates (Smith and Huyser, 1987). According to the 1993 and 1994 projected production statistics (USDA-FAS, 1994), approximately 42% of the total world's soybean production is planted in the United States, yielding 51% of the world production. The United States leads the world production in soybean. Together with Argentina, Brazil, and China, 87% of world production is accounted for (USDA-FAS, 1994).

Several herbicides are currently available to the grower for weed management in soybean. Weed management is critical for maximum soybean yield and is used on most soybean acreage grown in the United States. 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 when used at the application rates suggested for weed control.

Glufosinate-ammonium (GA), the active ingredient in Basta®, Ignite®, Rely® Liberty®, Harvest® and Finale™, is a broad spectrum, non-systemic, non-selective herbicide. It has very favorable environmental and safety features. Resistance to the herbicide has now been achieved, through the insertion of a resistance gene, in over 20 commercially important plant species including soybean. Genetically engineered Glufosinate Resistant Soybean (GRS) will provide a selective use for GA and a valuable new weed management tool to soybean producers.

For years pre-emergence herbicides have been the major tool used for weed control in conventional production. Entire fields were treated prior to, or at planting, and before the crop and weeds emerged. However, with the increase in no-tillage soybean and the advent of excellent post-emergence herbicides, a shift has occurred toward the treatment of weeds when and where they emerge. Applications may be made over the entire field, or as spot spraying, dependent on the weed density. GA, in concert with GRS, can positively impact current agronomic practices by participating in the shift toward the use of post-emergence herbicides which allows the grower to treat only as needed for weed control. AgrEvo believes that GRS offers the grower the choice and advantages of using a modern herbicide which features broad-spectrum weed control and favorable environmental features, such as low residual activity, low soil leaching, and low toxicity to nontarget organisms, to manage weeds in production fields.

II. The Soybean Family

A. History and Uses of Soybean

The primary center of soybean germplasm and initial domestication was in central China. This domestication occurred over many centuries and was highlighted during the Shang Dynasty about 1700-1100 B.C. During the period of strong emperors, the soybean remained only in China. As the Chinese dynasties began to degenerate, increased trading and emigration brought soybean germplasm out of China to other areas of Southeast and Southcentral Asia. These areas became the secondary center for soybean germplasm. These events occurred during the 1st through the 15-16th Century A.D. (Hymowitz and Newell, 1981)

In 1765 soybeans were introduced into Colonial America (Georgia) by a seaman, Samuel Bowen, who brought the seed back from the Orient. In 1770 Benjamin Franklin, while on a government mission in Europe, sent a friend in Pennsylvania some soybean seed collected in London. The crop, produced in Georgia, was utilized to manufacture soybean sauce and vermicelli (soybean noodles) (Hymowitz and Harlan, 1982).

Initial use of soybeans grown in America in the early 1800's was for the production of soy sauce. By the late 1800's soybeans were grown primarily as a forage crop. In 1904 work by George Washington Carver at the Tuskeegee Institute showed the nature of soybeans as a provider of protein and oil. A 60-pound bushel of soybeans yields about 48 pounds of protein and 11 pounds of oil. The eight essential amino acids needed in human nutrition and not produced by the body are found in soybean protein. Today soybeans is the second largest cash crop in the United States (American Soybean Association, 1994).

The switch in the growth of soybeans for grain instead of forage was initially brought about by use of its oil in the manufacture of soaps. By the mid-1930's defatted soybean meal had become an accepted protein concentrate in poultry and cattle feeds. Stabilization of the flavor in soybean oil gave it another impetus for use in human foods. Destruction of a great deal of cotton production by the boll weevil also gave reason for a second source of vegetable oil. By 1982-1983 76% of edible oil products were derived from soybeans compared to only 11% for the combined oils derived from cotton, corn and peanuts (Mounts et al., 1987). Soybean meal is a key source of animal feed protein concentrate. The great bulk of soybean meal is used in poultry feed (46%) and swine feed (32%). Only 9% each are used for beef and dairy cattle. The production of edible protein products is small when compared to the use of soybean meal as an animal feed concentrate (Mounts et al., 1987).

The uses of soybeans can be classified into oil products, whole soybean products and soybean protein products. Glycerol, fatty acids and sterols, are derived from soybean oil. Refined soy oil has many edible, technical and industrial uses. The same is true of lecithin, also derived from soybean oil. Whole soybeans are used to produce such items as soy sprouts, baked soybeans, roasted soybeans, full fat soy flour and the traditional soyfoods (miso, soymilk, soy sauce and tofu). Soybean protein products have a wide range of technical uses, edible uses and feed uses (American Soybean Association, 1994). While one might expect the use of superlatives by the organization, their use of the term "miracle crop" for soybeans may not be too far fetched.

B. Taxonomy of the Genus *Glycine*

Soybeans belong to the genus *Glycine*. This genus is a member of the *Leguminosae* (Legume) family, in the subfamily *Papilionoideae* and the tribe *Phaseoleae*. The genera of this tribe include some of the most economically important members of the entire legume family. They include such genera as *Glycine*, *Phaseolus* and *Vigna* (Hymowitz and Singh, 1987; Lackey, 1981).

The genus *Glycine* has been so broadly defined that it has included a range of 16-60 species. In 1962, F. J. Hermann, senior botanist with the USDA, conducted a very intensive survey of the genus and concluded it should consist of three subgenera with a total of eleven species. Following Hermann's designation of the genus *Glycine,* other taxonomists, in reviewing additional dried specimens and the literature, redesignated the genus so that it now only includes two subgenera, *Glycine* and *Soja*. The first consists of twelve wild perennial species (Hymowitz et al., 1992) that are primarily distributed in Australia, South Pacific Islands, Philippines, and Taiwan (Newell and Hymowitz, 1978). The subgenus *Soja* consists of three annual species from Asia, *G. max, G. soja,* and *G. gracilis*. The first species is the cultigen, the second species is the wild form of the soybean, and the third species is referred to as the "weedy" form of the soybean (Lackey, 1981). *Glycine gracilis* is known from Northeast China and is described as somewhat intermediate between *G. max* and *G. soja* (Skvortzov, 1927).

It is believed that *G. soja* is the wild ancestor of *G. max*. It is felt by some that the perennial nature is the primitive type and through evolution the wild annual and then the cultigen were produced. *Glycine soja* is distributed throughout China and into adjacent areas of the former USSR. It is also found in fields, hedgerows, along roadsides and on riverbanks in Japan, Korea and Taiwan (Hymowitz and Singh, 1987). The cultigen is grown on every continent.

The soybean plant first extends its two fleshy cotyledons above the soil surface. Characteristically the first true leaves of *Glycine max* are unifoliate. Subsequent

leaves are pinnately trifoliolate. These are arranged in an alternate fashion from nodes along the stem, while the two unifoliate leaves are arranged opposite to each other. Perfect flowers, containing both stamens and pistil, are formed on racemes in the axis of the leaves. The petals (corolla) include the large banner (standard) petal located in the posterior position with two lateral wing petals and two anterior keel petals which, while in close contact, are not fused. There is a single pistil which lies within nine elevated fused stamens with a single unattached stamen in the posterior position. This arrangement of the male and female organs within the same flower, and the fact that pollination occurs twenty-four hours prior to full flower opening, leads to an extremely high level of self-fertilization (Carlson and Lersten, 1987; Scott and Aldrich, 1970). This is an important point when discussing the genetics and breeding of soybeans.

C. Genetics of Soybean

The genus *Glycine* is unique within the legume family in that all species in the genus have a diploid number of 40 or 80 chromosomes but not 20. It is believed that diploid ancestors had a base number 11 which was reduced to 10 by loss of one chromosome. Polyploidy has produced the 40 and 80 somatic chromosome number. One might consider members of this genus to originally be regarded as tetraploids which, due to changes in genetic makeup, now act as diploids (Lackey, 1980; Singh and Horowitz, 1985).

Cultivated soybean is sexually compatible only with members of the genus *Glycine*. Under controlled conditions, intra - and interspecific subgeneric crosses have been made manually within the genus *Glycine*. Hybrid weakness, sterility, seedling lethality and seed inviability were found to occur in offspring of both types of crosses. In certain combinations pods began to abort within two to three weeks after pollination. In these cases *in vitro* culture techniques were used to complete the hybridization process and to germinate the immature seeds (Singh and Horowitz, 1985). These type of studies were conducted to see whether useful agronomic traits may be someday transferred to the cultivated soybean. Successful crosses can be made between the two annual species *G. max* and *G. soja*.

Soybeans are primarily a self-fertilized crop (see Section I.B. above). Soybean flowers contain both male and female reproductive organs within a small floral structure and whose petals fully open only after fertilization has occurred. Hybridization occurs, in nature at rates usually much less than 1.0% (Woodworth, 1922; Garber and Odland, 1926). It has been found that pollen movement within and between rows to male-sterile soybeans is very limited. A spacing between rows of at least 7-m and within rows of 12 to 18-m from the pollen source will result in little pollen contamination (Boerma and Moradshahi, 1975). The frequency of pollen transfer may be the limiting factor in soybean

cross-pollination rather than the failure of self-fertilization (Beard and Knowles, 1971). Some scientists believe that whatever small degree of natural hybridization takes place, bees and onion thrips play a role in cross-pollination. This may be a factor in explaining why very different genotypes, locations and seasons cause variation in natural hybridization (Weber and Hanson, 1961; Erickson, 1984). In fact, the future of hybrid soybean cultivars being developed to exploit any possible heterosis is dependent upon increasing cross-pollination in this crop plant. Significant problems still exist in both areas (Nelson and Bernard, 1984).

D. Weediness Potential of Soybean

Cultivated soybean has been domesticated worldwide for over 30 centuries and are grown on every continent (Agricultural Statistics, 1993). Its inherent agronomic characteristics, such as higher yield, resistance to pod shattering, plant height and resistance to lodging, have been intensified because soybean is naturally a self-fertilized plant. Soybeans, of the subgenus *Soja*, to which cultivated soybeans belong, are annuals reproducing only by seed. The seed does not remain viable for long periods of time. Collectively, these characteristics have reduced cultivated soybean chances of becoming a weed even though it has been intensively grown for many years over a wide region of the United States (USDA-APHIS, 1994; Rissler and Mellon, 1993). The USDA - APHIS (1994) has stated that cultivated soybean "is an annual crop and is considered to be highly domesticated, well characterized crop plant that is not persistent in undisturbed environments without human intervention." No reference on weed species lists the cultigen *G. max* or *G. gracilis* as a weed (USDA-APHIS, 1994). *Glycine soja*, however, is listed as a common weed in Japan by Holm et al. (1979), but is not listed in other texts of weeds found in Japan (Kasahara, 1982; Nemoto, 1982).

E. Potential for Outcrossing

The only wild species that are sexually compatible with the cultivated soybean are members of the genus *Glycine*. Most of these species are perennial plants in a different subgenus within the genus *Glycine*. The probability of gene transfer between the perennial wild species and cultivated soybeans in nature is very low. There are no known reports of natural hybridization between these two subgenera (Singh and Hymowitz, 1985; Newell and Hymowitz, 1978; USDA-APHIS, 1994). Only one wild annual species, *Glycine soja,* is compatible with the cultivated soybean. No wild relatives can be found within the continental United States, although some wild perennials are found in the South Pacific territories of the United States (USDA-APHIS, 1994; Rissler and Mellon, 1993). Since there are no perennial or wild annual species of *Glycine* in the United States, outcrossing, if it were to occur, would be limited to other cultivated soybean.

In all discussions on potential outcrossing, it must be kept in mind that soybeans are a self-fertilized plant and its pollen is not spread to any degree by the wind. There is also little data to show that bees, while sometimes aggressively foraging in soybeans, are an important vector in pollen transfer. In fact, Certified Seed Regulations (7 CFR 201.76-201.78) acknowledge this low possibility of cross pollination in the safeguards set up for Foundation, Registered and Certified seed. The most stringent regulations exist in the production of Foundation seed, where blocks of soybeans of different lines can be grown adjacent to one another, as long as the distance is sufficient to prevent contamination by mechanical (not pollination) mixing.

III. The Transformation System and Plasmid Used

The GRS transformation events W62 and W98 contain the native *bar* and the *gus* genes derived from *Streptomyces hygroscopicus* (Thompson et al, 1987) and *Escherichia coli* (Jefferson et al., 1986), respectively. The *bar* gene is fused to a *35*S promoter from CaMV, an AMV leader sequence, and the nontranslated 3' region of the ribulose-1,5-bisphosphate carboxylase (RuBP) small subunit (SSU) gene from *G. max*. The *gus* gene is fused to the *35*S promoter, the AMV leader sequence, and the nontranslated 3' region of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens*. Both genes are contained in the plasmid pWRG2114. The events A2704-12, A2704-21 and A5547-35 contain a synthetic version of the *pat* gene derived from *Streptomyces viridochromogenes*, strain Tü 494 (Bayer et al., 1972). 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 synthetic *pat* gene is fused to a *35*S promoter and terminator from CaMV forming a *pat* gene cassette. The gene is contained in the plasmid pB2/35SAcK. Both the *bar* and *pat* genes encode the enzyme phosphinothricin acetyltransferase (PAT), which confers resistance to the herbicide GA. The *gus* gene encodes the enzyme β-glucuronidase. The plasmids used to transform the parental lines contain no other plant expressible genes. The plasmids were transferred to the soybean genome using the particle acceleration method (particle gun). Stable insertion of the *bar* or *pat* gene into the soybean genome results in the expression of the PAT enzyme.

A. Particle Acceleration Transformation System

Agracetus, Inc., Middleton, Wisconsin, introduced the plasmid DNA into soybean tissue by the particle acceleration method as described previously (McCabe et al., 1988; Christou et al., 1988). In this method DNA is precipitated onto microscopic gold or tungsten particles. The coated particles are spread onto a mylar carrier sheet which is then accelerated towards a stainless steel retaining screen. The screen stops the flight of the sheet but allows the continued flight of the DNA coated particles. The particles penetrate the target plant cells where the DNA is deposited and introduced into the cell genome. The cells are induced to produce shoots on plant tissue culture medium containing plant hormones. The shoots which develop from the transformed cells express the phenotype encoded by the genes on the introduced DNA. The expression of the introduced genes is used as evidence of transformation. Expression of the *gus* marker gene is detected by a staining method in which the GUS enzyme converts a substrate into a blue precipitate. Plant tissue which produces the blue color after the histochemical reaction is expressing the *gus* gene. Expression of the PAT enzyme is detected by spraying plantlets in axenic culture with GA. Surviving plantlets are transferred to soil, grown in the greenhouse and then screened again for glufosinate resistance.

B. Parent Lines

Asgrow Seed Company, Kalamazoo, Michigan, *Glycine max* cultivars A5403 and A3322 were used for transformation, resulting in primary transformation events W62 and W98, respectively. A5403 is a maturity group V cultivar of the bushy plant type, with purple flowers, gray pubescence, imperfect black hilum and tan pod wall color. It combines high yield, good standability, excellent emergence, resistance to races 3 and 14 of the soybean cyst nematode, and tolerance to many leaf and stem diseases. A3322 is a maturity group III cultivar of the bushy plant type, with white flowers, tawny pubescence, black hilum color and tan pod wall color. It combines high yield, good standability, excellent emergence, and tolerance to many leaf and stem diseases. It contains the Phythopthora root rot resistance gene *Rps1c*. Transformation events W62 and W98 have been crossed with Asgrow elite lines.

Asgrow Seed Company *Glycine max* cultivars A2704 and A5547 were used for transformation, resulting in primary transformation events A2704-12, A2704-21, and A5547. A2704 is a maturity group II cultivar of the intermediate plant type, with purple flowers, tawny pubescence, black hilum color and tan pod wall color. It combines high yield, good standability, excellent emergence, and tolerance to many leaf and stem diseases. It contains the Phythopthora root rot resistance gene *Rps1k*. It was selected for tolerance to sulfonyluera herbicides following chemical mutagenesis. A5547 is a maturity group V cultivar of the intermediate plant type, with white flowers, gray pubescence, buff hilum color and tan pod wall color. It combines high yield, good standability, excellent emergence, resistance to races 3 and 14 of the soybean cyst nematode, and tolerance to many leaf and stem diseases. Transformation events A2704-12, A2704-21, and A5547 have been crossed with Asgrow elite lines.

The commercialization strategy for GRS is to use traditional backcrossing and breeding to transfer the glufosinate resistance locus from the transformation events to a wide range of varieties with a wide range of maturities.

C. Construction of Plasmids Used

The plasmid pWRG2114, also known as pCMC2114, was used to transform the parental lines A5403 and A3322 to generate primary transformation events W62 and W98, respectively. This plasmid is a derivative of the high copy *E. coli* plasmid pUC19 (Yanisch-Perron et al., 1985). It contains two plant expressible genes, the *bar* gene and the *gus* gene, in opposite orientation. The *bar* gene is fused to a *35*S promoter from CaMV, an AMV leader sequence, and the nontranslated 3' region of the RuBP carboxylase SSU gene from *G. max*. The *gus* gene is fused to the *35*S promoter, the AMV leader sequence, and the nontranslated 3' region of the nopaline synthase (*nos*) gene from *A. tumefaciens*. The chimeric *bar* gene can be isolated as a 1363 bp *Xba*I

fragment. The chimeric *gus* gene can be isolated as a 1880 bp *NcoI/SmaI* fragment.

The plasmid pB2/35SAcK, also known as pWRG5143, was used to transform the parental lines A2704 and A5547 to generate the primary transformation events A2704-21, A2704-21, and A5547-35. To construct pB2/35SAcK, the synthetic *pat* gene was cloned into the *Sal*1 site, between the CaMV derived *35S* gene promoter and terminator sequences, of the pUC derived plasmid pDH51 (Pietrzak et al., 1986). The chimeric *pat* gene cassette (*35S* promoter::*pat*::*35S* terminator) was then isolated as a 1.3 kb *Eco*R1 fragment and cloned into pUC19 (Yanisch-Perron et al., 1985). The construct contains no other plant expressible genes.

The pUC sequences in both plasmids include an ampicillin resistance (*ampR)* gene and a bacterial origin of replication. The *ampR* gene has regulatory signals recognized in bacteria but not functional in transgenic soybean cells. Plasmid pB2/35SAcK also contains the Right Border of the Ti octopine plasmid B6S3 which was obtained from pTiAch5 (Gielen et al., 1984) and inserted into the singular *Nde*1 site.

The complete sequences of pWRG2114 and pB2/35SAcK are shown in Appendix 1. A map of the pWRG2114 vector is shown in Figure III.1., and a map of the pB2/35SAcK vector is shown in Figure III.2. A comparison of the native *pat* nucleotide sequence with that of the synthetic sequence is shown in Figure.III.3. A comparison of the native *bar* nucleotide sequence with that of the synthetic *pat* sequence is shown in Figure.III.4. A comparison of the PAT proteins is shown in Figure III.5. Descriptions of the DNA elements in pWRG2114 and pB2/35SAcK are shown in Tables III.1. and III.2., respectively.

D. Open Reading Frames and Associated Regulatory Regions

Although pWRG2114 contains three open reading frames, *ampR* , *gus* and *bar*, only the *bar* and *gus* reading frames are intact and functional in transformation events W62 and W98, as will be shown in Section IV. Vector pB2/35SAcK contains two open reading frames, *ampR* and *pat.* Only the *pat* reading frame is functional and intact in the events A2704-12, A2704-21 and A5547-35. The GRS transformation events W62 and W98 have been considered regulated articles because they contain DNA sequences from *A. tumefaciens*, AMV, and CaMV, organisms which are considered to be plant pests. The events A2704-12, A2704-21 and A5547-35 are considered regulated articles because they contain DNA sequences from CaMV and *A. tumefaciens.* This section contains a more thorough description of the inserted genetic material responsible for expression of the glufosinate resistance trait. The *ampR* and *gus* genes are also addressed. Refer to Tables III.1. and III.2. for a description of all other introduced genetic sequences in pWRG2114 and pB2/35SAcK, respectively.

1. CaMV *35S* promoter and terminator The *35S* promoter and terminator sequences are derived from CaMV (Odell et al., 1985). The promoter controls transcription initiation of the *bar, gus* and *pat* genes. The terminator ends transcription of the *pat* gene. CaMV is a doublestranded DNA caulimovirus with a host range restricted primarily to cruciferous plants. The *35S* promoter directs high level constitutive expression and is widely used as a promoter for high expression of genes (Harpster et al., 1988). The CaMV sequences, as used in the GRS, do not cause the soybean to become a plant pest.

2. AMV leader sequence The AMV leader sequence is derived from the AMV RNA 4 transcript where it provides a 5'-terminal cap structure for the transcript thereby facilitating the initiation of translation (Gehrke et al., 1983). The leader sequence is between the promoter and coding sequences of the *bar* and *gus* genes. The AMV sequences, as used in the GRS, do not cause the soybean to become a plant pest.

3. *nos* and RuBP carboxylase SSU termination sequences The 3' nontranslated region from the *nos* and RuBP carboxylase SSU genes provide the site for transcription termination for the chimeric *gus* and *bar* genes. respectively. The *nos* gene termination sequences are derived from the T-DNA of the nopaline Ti plasmid pTiT37 harbored by a strain of *A. tumefaciens* (Depicker et al., 1982). The *nos* gene encodes nopaline synthase, which, in crown gall cells, catalyzes the formation of nopaline by the reductive condensation of α-ketoglutaric acid and arginine (Ellis and Murphy, 1981). The *nos* sequences, as used in the GRS, do not cause the soybean to become a plant pest. The RuBP carboxylase SSU termination sequences are derived from the SRS1 gene found in the nuclear genome of *G. max* (Berry-Lowe et al., 1982). The RuBP carboxylase enzyme is comprised of both small and large subunits. It is one of the most abundant proteins in nature and catalyzes the first step in the Calvin cycle, a cycle responsible for converting fixed carbon into more utilizable sugars in plant cells.

4. *bar* and *pat* The *bar* gene is the native gene isolated from *S. hygroscopicus* (Thompson et al, 1987). The *pat* gene is a synthetic version of the *pat* gene isolated from *S. viridochromogenes*, strain Tü 494 (Bayer et al., 1972). 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 (Figure III.3.). The nucleotide sequences of the *bar* and synthetic *pat* genes share 65.6% homology (Figure III.4.). Both the *bar* and *pat* genes encode the enzyme phosphinothricin acetyltransferase (PAT), which imparts resistance to the phytotoxic activity of GA. The PAT enzyme derived from these genes share 84.7% homology (Figure III.5.).

Members of the genus *Streptomyces* are gram-positive sporulating soil bacteria. These organisms synthesize numerous unique compounds, secondary metabolites, that often possess antibacterial, antitumor, or antiparasitic activity (Demain et al., 1983). One such compound, the antibiotic bialaphos, is produced by both *S. viridochromogenes* and *S. hygroscopicus*. Bialaphos (syn. L-phosphinothricyl-L-alany-L-alanine) is an herbicidally active tripeptide consisting of two L-alanine molecules and an analog of L-glutamic acid called phosphinothricin. When it is released by peptidases, the L-PPT moiety , is a potent inhibitor of GS (Bayer at el. 1972). L-PPT is the active component of the commercial herbicides, Herbiace® (Meiji Seika Ltd.) and Basta®, Ignite®, Rely® Liberty® and Harvest® and Finale™ (AgrEvo GmbH). Herbiace® is bialaphos that is commercially produced using *S. hygroscopicus*. The other herbicides are the ammonium salts of phosphinothricin, common name GA, and are chemically synthesized.

L-PPT is a potent inhibitor of the enzyme GS in both bacteria and plants, where it apparently binds competitively to the enzyme by displacing L-glutamate from the active site. Evidently GS binds L-PPT better than the substrate. GS plays a central role in nitrogen metabolism of higher plants where it is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation and photorespiration (Miflin and Lea, 1976). Ammonia, although a plant nutrient and metabolite, is toxic in excess and leads to death of plant cells (Tachibana et al., 1986).

Although the GS from both *S. viridochromogenes* and *S. hygroscopicus* are sensitive to L-PPT, the bacteria produce an inactivating enzyme, PAT. PAT catalyzes the conversion of L-PPT to N-acetyl-L-PPT in the presence of acetyl CoA as a co-substrate. N-acetyl-L-PPT does not inactivate GS, and, thus, has no herbicidal activity. Therefore, plants expressing the PAT enzyme are resistant to the phosphinothricin class of herbicides. The PAT enzyme is encoded by the *bar* (*b*ialaphos-*r*esistance) gene in *S. hygroscopicus*, and by the *pat* gene in *S. viridochromogenes*. These genes function both as an integral part of the biosynthetic pathway of bialaphos and as an enzyme which confers resistance (Kumada, 1986).

5. *gus* The *gus* gene, also known as the *uidA* gene, is derived from *E. coli* (Jefferson et al., 1986). It encodes the enzyme β-glucuronidase and is widely used as a scorable marker gene in plant transformation since many higher plants contain no detectable β-glucuronidase activity (Jefferson et al., 1987). Expression of the *gus* marker gene is detected by a histochemical staining method in which the enzyme converts a colorless substrate into a blue precipitate. β-glucuronidase genes and enzymes are found throughout nature (Fishman, 1955; Paigen, 1989).

6. *ampR* The ampicillin resistance gene was isolated from pBR322, a plasmid of *E. coli* (Sutcliffe, 1978). It encodes a β-lactamase. β-lactamase genes are found throughout nature (Sykes and Smith, 1979) . The gene is expressed in bacteria where it is used in the selection of transformed bacteria which are then used to amplify the plasmid vector.

Table III.1. Genetic Elements of the Vector pWRG2114

Genetic element	Position in vector	Size (Kb)	Function
ampR	201-1062	0.86	Ampicillin resistance gene from *E. coli* expresses a ß-lactamase only in bacteria. (Sutcliffe, 1978)
ori-pUC	1822-1269	0.55	Origin of replication (ColE1) of pUC19 (Yanisch-Perron et al., 1985).
P-35S	2235-2684; 5796-6230	0.43	The CaMV promoter of the *35S* transcript (Odell et al., 1985).
AMV leader	2685-2720; 5795-5760	0.035	The AMV leader sequence of the RNA 4 transcrip (Gehrke et al., 1983).
gus	2721-4532	1.81	The β-glucuronidase coding region from *E. coli* (Jefferson et al., 1987).
bar	5760-5206	0.55	The glufosinate resistance gene coding region fro *S. hygroscopicus* (Thompson et al., 1987.
T-*nos*	4600-4857	0.26	The 3' nontranslated region from the *A. tumefacie* nopaline synthase gene (Depicker et al., 1982).
T-SSU	5185-4886	0.32	The 3' nontranslated region from the *G. max* ribulose-1,5-bisphosphate carboxylase small subu gene (Berry-Lowe et al., 1982).

Table III. 2. Genetic Elements of the Vector pB2/35SAcK

Genetic element	Position in vector	Size (Kb)	Function
RB	189-243	0.054	Right border sequence of *A. tumefaciens* Ti plasmi pTiAch5 (Gielen et al., 1984).
P-35S	461-1003	0.54	The CaMV promoter of the *35S* transcript. (Odell et al., 1985)
pat	1012-1563	0.55	The synthetic glufosinate resistance gene. (Eckes et al., 1989)
T-35S	1582-1784	0.20	The CaMV 3´-nontranslated region of the *35S* transcript (Pietrzak et al., 1986).
ori-pUC	2253-2803	0.55	Origin of replication (ColE1) of pUC18. (Yanisch-Perron et al., 1985)
ampR	3876-3016	0.86	Ampicillin resistance gene from *E. coli* expresses a ß-lactamase only in bacteria (Sutcliffe, 1978).

Figure III.1. Vector Map of pWRG2114

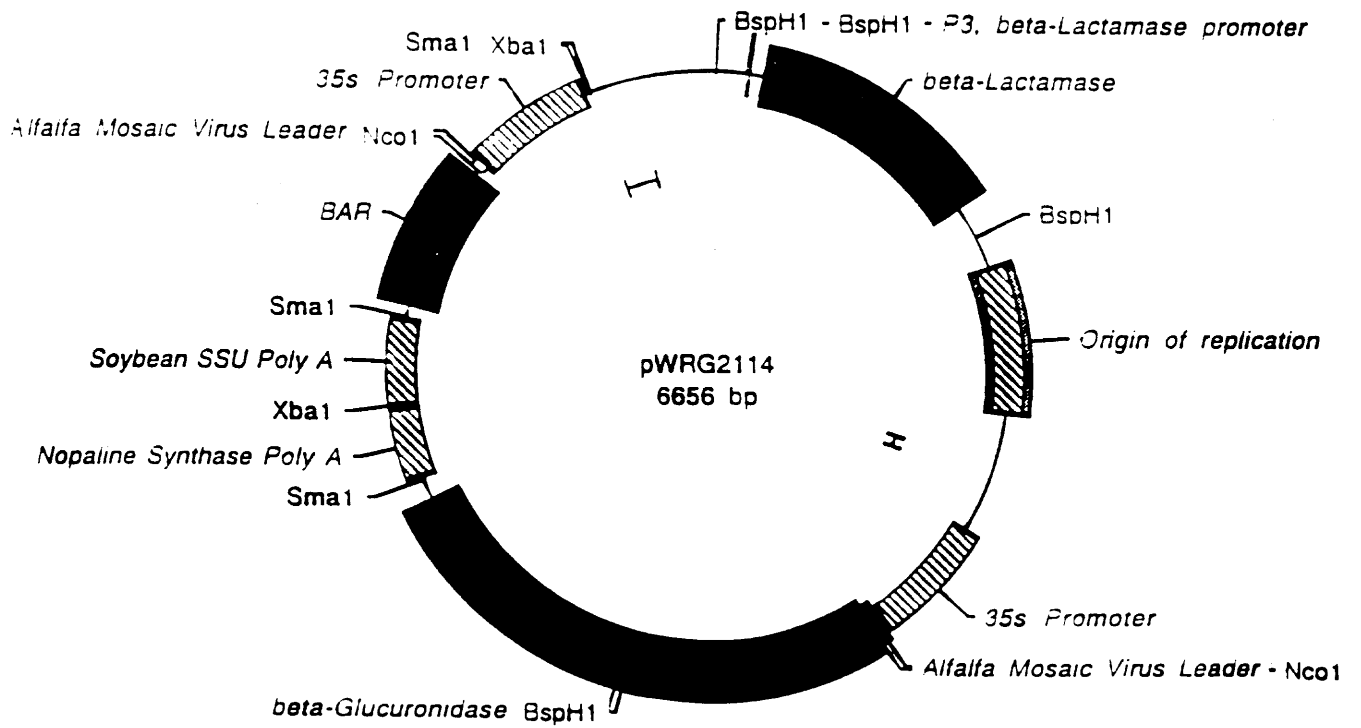


Figure III.2. Vector Map of pB2/35SAcK

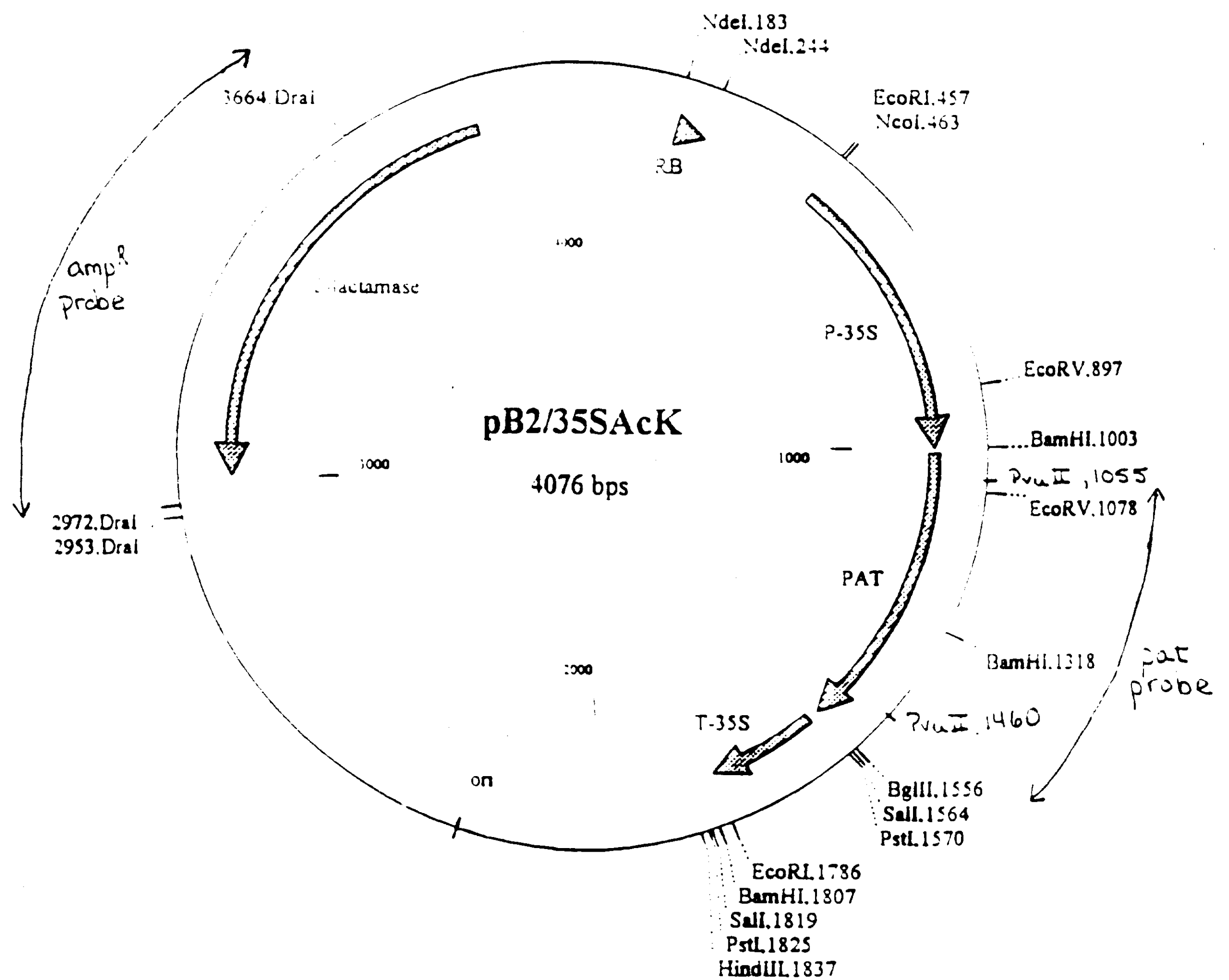


Figure III.3. Comparison of the synthetic *pat* nucleotide sequence (capital letters) with that of the native *pat* sequence (small letters). The sequences have 70% homology.

```
  1 ATGTCTCCGGAGAGGAGACCAGTTGAGATTAGGCCAGCTACAGCAGCTGA  50
  1 gtgagcccagaacgacgcccggtcgagatccgtcccgccaccgccgccga  50

 51 TATGGCCGCGGTTTGTGATATCGTTAACCATTACATTGAGACGTCTACAG 100
 51 catggcggcggtctgcgacatcgtcaatcactacatcgagacgagcacgg 100

101 TGAACTTTAGGACAGAGCCACAAACACCACAAGAGTGGATTGATGATCTA 150
101 tcaacttccgtacggagccgcagactccgcaggagtggatcgacgacctg 150

151 GAGAGGTTGCAAGATAGATACCCTTGGTTGGTTGCTGAGGTTGAGGGTGT 200
151 gagcgcctccaggaccgctacccctggctcgtcgccgaggtggagggcgt 200

201 TGTGGCTGGTATTGCTTACGCTGGGCCCTGGAAGGCTAGGAACGCTTACG 250
201 cgtcgccggcatcgcctacgccggcccctggaaggcccgcaacgcctacg 250

251 ATTGGACAGTTGAGAGTACTGTTTACGTGTCACATAGGCATCAAAGGTTG 300
251 actggaccgtcgagtcgacggtgtacgtctcccaccggcaccagcggctc 300

301 GGCCTAGGATCCACATTGTACACACATTTGCTTAAGTCTATGGAGGCGCA 350
301 ggactgggctccacccctacacccacctgctgaagtccatggaggccca 350

351 AGGTTTTAAGTCTGTGGTTGCTGTTATAGGCCTTCCAAACGATCCATCTG 400
351 gggcttcaagagcgtggtcgccgtcatcggactgcccaacgacccgagcg 400

401 TTAGGTTGCATGAGGCTTTGGGATACACAGCCCGGGTACATTGCGCGCA 450
401 tgcgcctgcacgaggcgctcggatacaccgcgcgcgggacgctgcgggca 450

451 GCTGGATACAAGCATGGTGGATGGCATGATGTTGGTTTTTGGCAAAGGGA 500
451 gccggctacaagcacgggggctggcacgacgtggggttctggcagcgcga 500

501 TTTTGAGTTGCCAGCTCCTCCAAGGCCAGTTAGGCCAGTTACCCAGATCT 550
501 cttcgagctgccggccccgccccgccccgtccggcccgtcacacagatcc 550

551 GA 552
551 ga 552
```

Figure III.4. Comparison of the synthetic *pat* nucleotide sequence (small letters) with that of the native *bar* sequence (capital letters)

```
pat.dna    atgtctccggagaggagaccagttgagattaggccagctacagcagctgatatggccgcg
           :::   :: ::  :  : :: :  :: ::  : :  :: :: :  :: :: ::: : :::
BAR.DNA    ATGAGCCCAGAACGACGCCCGGCCGACATCCGCCGTGCCACCGAGGCGGACATGCCGGCG

pat.dna    gtttgtgatatcgttaaccattacattgagacgtctacagtgaactttaggacagagcca
           :: ::    ::::: ::::: ::::: :::::    :: :: :::::  : :: :::::
BAR.DNA    GTCTGCACCATCGTCAACCACTACATCGAGACAAGCACGGTCAACTTCCGTACCGAGCCG

pat.dna    caaacaccacaagagtggattgatgatctagagaggttgcaagatagataccccttggttg
           ::   ::: :: :::::::  :: :: :: :   :  :::  ::  : :: :: ::: :
BAR.DNA    CAGGAACCGCAGGAGTGGACGGACGACCTCGTCCGTCTGCGGGAGCGCTATCCCTGGCTC

pat.dna    gttgctgaggttgagggtgttgtggctggtattgcttacgctgggccctggaaggctagg
           :: :: ::::: :: :: :  :: :: :: :: :: ::::: :: :::::::::::  :
BAR.DNA    GTCGCCGAGGTGGACGGCGAGGTCGCCGGCATCGCCTACGCGGGCCCCTGGAAGGCACGC

pat.dna    aacgcttacgattggacagttgagagtactgtttacgtgtcacataggcatcaaaggttg
           ::::: ::::: ::::: :  :::   :: :: ::::: :: :   : :: ::  ::  :
BAR.DNA    AACGCCTACGACTGGACGGCCGAGTCGACCGTGTACGTCTCCCCCCGCCACCAGCGGACG

pat.dna    ggcctaggatccacattgtacacacatttgcttaagtctatggaggcgcaaggttttaag
           :: :: :: :::::  : ::::: ::  :::: :::::  ::::::: :: :: :: :::
BAR.DNA    GGACTGGGCTCCACGCTCTACACCCACCTGCTGAAGTCCCTGGAGGCACAGGGCTTCAAG

pat.dna    tctgtggttgctgttataggccttccaaacgatccatctgttaggttgcatgaggctttg
           ::::: ::::: :: :: :: :: ::::: ::    :: :  :  :::: :::::  :
BAR.DNA    AGCGTGGTCGCTGTCATCGGGCTGCCCAACGACCCGAGCGTGCGCATGCACGAGGCGCTC

pat.dna    ggatacacagcccggggtacattgcgcgcagctggatacaagcatggtggatggcatgat
           :::::  :  :::: :: :   :::: :: :: :: : :::::: ::     ::::::::
BAR.DNA    GGATATGCCCCCCGCGGCATGCTGCGGGCGGCCGGCTTCAAGCACGGGAACTGGCATGAC

pat.dna    gttggtttttggcaaagggattttgagttgccagctcctccaaggccagttaggccagtt
           :: ::::: :::::   ::: ::     :::: :  :: ::  : :: ::   ::: ::
BAR.DNA    GTGGGTTTCTGGCAGCTGGACTTCAGCCTGCCGGTACCGCCCCGTCCGGTCCTGCCCGTC

pat.dna    acccagatctga
           ::: ::::::::
BAR.DNA    ACCGAGATCTGA
```

Nucleotide sequences have 65.6% homology.

Figure III.5. Comparison of the *pat* amino acid sequence (small letters) with that of the *bar* sequence (capital letters)

```
pat.pro     msperrpveirpataadmaavcdivnhyietstvnfrtepqtpqewiddlerlqdrypwl
            :::::::  :: :: ::: ::: :::::::::::::::::: :::: ::: ::  :::::
BAR.PRO     MSPERRPADIRRATEADMPAVCTIVNHYIETSTVNFRTEPQEPQEWTDDLVRLRERYPWL

pat.pro     vaevegvvagiayagpwkarnaydwtvestvyvshrhqrlglgstlythllksmeaqgfk
            :::: : ::::::::::::::::::: ::::::: :::: ::::::::::::: ::::::
BAR.PRO     VAEVDGEVAGIAYAGPWKARNAYDWTAESTVYVSPRHQRTGLGSTLYTHLLKSLEAQGFK

pat.pro     svvaviglpndpsvrlhealgytargtlraagykhggwhdvgfwqrdfelpapprpvrpv
            :::::::::::::::.::::::  :: ::::: ::: :::::::: :: :: ::::: ::
BAR.PRO     SVVAVIGLPNDPSVRMHEALGYAPRGMLRAAGFKHGNWHDVGFWQLDFSLPVPPRPVLPV

pat.pro     tqi
            : :
BAR.PRO     TEI
```

Amino acid sequences have 84.7 % homology

IV. Genetic Characterization of Transformation Events W62 and W98

A. Description, History and Mendelian Inheritance

Transformation events W62 and W98

Primary transformation events W62 and W98 are derived from the transformation cultivars A5403 and A3322, respectively, as described in Section III. These were backcrossed to their respective parent cultivars for several generations before crossing with proprietary lines. Through traditional breeding with these fertile transformation events individuals homozygous at the *bar* locus have been produced. Traditional backcrossing and breeding will be used to continue to transfer the glufosinate resistance locus in events W62 and W98 to a wide range of soybean varieties with a wide range of maturities.

Transformation events W62 and W98 have been field tested by AgrEvo USA Company and Asgrow Seed Company since 1990 in the primary soybean growing regions of the United States, including Puerto Rico. These tests have occurred at approximately 197 sites under field release authorizations granted by APHIS (USDA authorizations: permits 90-274-05, 91-051-03, 91-203-01, 92-043-02, 92-043-03, 93-090-01, 93-047-02, 93-047-03; notifications 93-120-31, 93-120-35, 93-127-02, 93-270-03, 94-080-03, 94-090-02, 94-131-01, 95-034-02, 95-069-01, 95-069-02, 95-069-03, 95-069-04, 95-069-05, 95-069-06, 95-069-07, 95-069-08, 95-069-09, 95-069-10, 95-069-11, 95-069-12, 95-079-02, 95-115-04, 95-135-04, 95-142-02). Transformation events W62 and/or W98 have 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 GA. In these trials, when sprayed with the herbicide, all plants exhibited a high level of glufosinate resistance, indicating that the gene is stably integrated and expressed.

The *bar* locus has been stabilized in W62 and W98 homozygotes for several generations. To incorporate these transformation events the original hemizygous transformed plants were self-pollinated. This resulted in progeny segregating in a 3:1 fashion with respect to glufosinate resistance. Resistant progeny were selected from a population of young soybean plants by spraying with GA. These homozygous or hemizygous resistant individuals were again self-pollinated producing progeny which segregated 3:1 with respect to glufosinate resistance. The resistant progeny were either homozygous or hemizygous for the *bar* locus. Homozygous plants were those from which all progeny from the 2nd self-pollination were unharmed by GA. The seed from the homozygous plants were again self-pollinated and the progeny were sprayed with GA. If the *bar* locus is stable, then all progeny should be resistant to GA, as has been the case for some sublines with successive self-pollinations for additional generations. Further evidence supporting stable integration is shown

by Southern blot analysis of several generations of W62 and W98 (See Section IV.B.3).

Stability has been examined by evaluating the segregation of the glufosinate resistance phenotype in self-pollination of hemizygous or homozygous transformation events W62 and W98. Mendelian inheritance of the *bar* locus as a single dominant locus in transformation events W62 and W98 has not been consistently confirmed in segregation trials in the field (See termination reports 90-274-05, 93-047-02, and 94-090-02 in Appendix 2.). This is in contrast to observations from efficacy trials where all individuals derived from the GRS events W62 and W98 have been resistant when treated with GA (See other termination reports in Appendix 2).

The lack of fit to the single dominant gene model in segregation trials may be due to two factors. During a previous generation all susceptible plants may not have been eliminated due to incomplete spraying with GA. In this case, susceptible progeny would have been collected at harvest, planted, and evaluated in the most recent generation. A second factor may be the result of classifying rows in the most recent generation as heterozygous if even one plant appeared injured and dying when evaluated after GA treatment. This sporadic plant death may have been due to disease organisms that were not recognized.

<u>Transformation events A2704-12, A2704-21 and A5547-35</u>

Primary transformation events A2704-12, A2704-21, and A5547-37 are derived from the cultivars A2704 and A5547 as described in Section III. These were backcrossed to their respective parent cultivars for several generations and will be crossed with proprietary lines in the near future. Through traditional breeding with these fertile transformation events individuals homozygous at the *pat* locus have been produced. Traditional backcrossing and breeding will be used to continue to transfer the glufosinate resistance locus in events A2704-12, A2704-21, and A5547-37 to a wide range of soybean varieties with a wide range of maturities.

Events A2704-12, A2704-21 and A5547-35 were evaluated in the field in 1995 at 2 sites (Maryland and Puerto Rico) under authorizations granted by APHIS (95-034-02 and 95-122-03). The purpose of the trials was to increase seed, advance generations, demonstrate the agronomic performance, and/or to evaluate segregation ratios of these additional events.

The *pat* locus has been stabilized in A2704-12, A2704-21 and A5547-35 homozygotes for some generations. To incorporate the *pat* gene into these transformation events the original hemizygous transformed plants were self-pollinated. The GA resistant progeny was again self-pollinated. This resulted in R2 progeny segregating in a 3:1 fashion with respect to glufosinate resistance (Table IV.1), the expected segregation ratio for a single dominant *pat* locus. This was not apparent when pooled A2704 and A5547 transformation events

were evaluated (See termination report 95-122-03 in Appendix 2.). Resistant R2 progeny were selected from a population of young soybean plants by spraying with GA. Seed (R3) from homozygous or hemizygous resistant individuals of A2704 events were planted. Progeny rows segregated 2:1 (entire rows resistant : partial rows resistant) with respect to glufosinate resistance (Table IV.1). The progeny from fully resistant rows were homozygous for the *pat* locus while those from partially resistant rows were hemizygous for the locus. Homozygous plants were those from which all progeny from the 2nd self-pollination were unharmed by GA. If the *pat* locus is stable, then all progeny should be resistant to GA. This will be evaluated during subsequest growing seasons.

Table IV.1. Segregation Data for Individuals and Rows of Progeny of Self-pollinated Events A2704-12, A2704-21, and A5547-35

Event	Progeny[a]	Resistant	Sensitive	Expected Ratio	χ^2 [b]
A2704-12	R2	67	24	3:1	0.10
	R3	24	45	2:1	0.06
A2704-21	R2	131	48	3:1	0.31
	R3	34	93	2:1	2.46
A5547-35	R2	63	30	3:1	2.61

[a] R2 = segregation of individual progeny from self-pollination of event; R3 = segregation of entire versus partially resistant rows derived from resistant R2 progeny.

[b] No significant difference (p=0.05) for the Chi square goodness-of-fit test for hypothesis of either 3:1 or 2:1 segregation. (Significance at p=0.05 for $\chi^2 \geq$ 3.84, df = 1).

B. DNA Analysis of Glufosinate Resistant Soybean

To determine the nature, number and molecular stability of insertions which occurred in the transformation events, Southern hybridization was used. Southern analysis was used to estimate the copy number of the insertions, the stability of these insertions over several generations and to map the inserted DNA at a basic level. Stability over several generations was determined only for events W62 and W98.

1. Copy Number and Insertion Integrity

Experiments were performed to determine the number of copies and insertion integrity of the *bar, gus* and *ampR* genes present in progeny of transformation events W62 and W98, and of the *pat* and *ampR* genes present in progeny of transformation events A2704-12, A2704-21, and A5547-35. When transforming a plant with restriction digested or intact, circular vector DNA there is no way to predict at which site or sites on the vector recombination will initiate. We have therefore used Southern blot analyses to examine the integrity of the inserted vector in GRS transformation events. These analyses also serve to determine the copy number of the inserted genes.

Transformation events W62 and W98

In the experiments restriction digested genomic DNA from transgenic plants homozygous for the integrated DNA were run in parallel with a dilution set of digested pWRG2114 vector on an agarose gel. After blotting and hybridization with a *bar-*, *gus-* or an *ampR-* probe the number of copies (intact and partial) of the genes in the soybean genome were quantified by comparing the hybridization intensity of the soybean DNA with the hybridization intensity of the diluted transformation vector. Such reconstruction experiments can only give a rough estimate of the copy number since the parameters for calculation (mass of soybean genome, spectrophotometric quantification of vector and plant DNA, dilution of DNAs, visual comparison of band intensity) are not absolutely precise.

DNA was digested with *XbaI* (for elucidation of the *bar, gus* and *ampR* genes) or *BspH1* (for elucidation of the *ampR* gene). See Figure III.1. to locate restriction sites in pWRG2114. After separation of the DNA by electrophoresis, the DNA was transferred to a nylon membrane and hybridized with a ^{32}P-labeled Polymerase Chain Reaction (PCR) generated *bar* gene fragment (442 bp fragment) (Figure IV. 1), with a ^{32}P-labeled *gus* gene fragment (1880 bp *Nco1/Sma1* fragment) (Figure IV. 2), or with a ^{32}P-labeled *ampR* gene fragment (1008 bp *BspH1* fragment) (Figure IV. 3, 4 and 5). The primers used to generate the *bar* fragment are 20mers located internal to the gene. Lanes contain 10 ug of restricted DNA. The amount of restricted pWRG2114 in positive control lanes is equivalent to 1.0 and 0.2 copies of the plasmid integrated in 10 ug of soybean DNA. The probed membrane was visualized by both autoradiography and by PhosphoImager™ (Molecular Dynamics).

The hybridizing fragments expected and observed when using the *bar, gus* or *ampR* gene as probe are listed in Table IV. 2.

Table IV. 2. Hybridizing Fragments in Southern Blots of W62 and W98 DNA Probed with the *bar*, gus, or *ampR* Gene

Probe - Restriction Enzyme	Expected Fragment (kb)[a]	Observed Fragment (kb)	
		W62	W98
bar - *Xba*1	1.3	1.3, > 2.3	1.3; 5 bands >2.0
gus - *Xba*1	5.3	2 bands < 4.0;1.3, 6.6	1.3; ~11 other bands
ampR - *Xba*1	5.3	3 bands < 5.0; 5.3	5.3; ~11 other bands
ampR - *BspH*1	1.0	1.0, > 9.0	1.0, > 4.4

[a] Expected fragment sizes for 1 copy of inserted vector.

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

In Figure IV. 1a. the intensity of the *bar* band (1.3 kb) in the W62 digests (lanes 1-5) is about double the signal intensity of lane 9 (1 copy). This suggests there are probably two copies of the *bar* gene present in these plants. The larger minor band suggests that a second locus contains the *bar* gene or a fragment of the gene exists within the genome. This interpretation is based on the size difference and weaker hybridization pattern. The probe did not hybridize to DNA from nontransgenic soybean (lanes 6-7).

In Figure IV. 1b. the intensity of the *bar* band (1.3 kb) in the W98 digests (lanes 2-7) is considerably more intense relative to the signal intensity of lane 14 (1 copy). The unexpected larger bands suggest that a fragment or intact copy of the *bar* gene exists at several different loci within the genome. There appears to be at least a total of 6 different loci where the plasmid, or a plasmid fragment containing the *bar* gene inserted into the genome. The probe did not hybridize to DNA from nontransgenic soybean (lanes 8-11).

In Figure IV. 2.a. the W62 digests (lanes 3-7) show prominent hybridization of the *gus* band at the expected size (5.3 kb) and weaker hybridization bands that are slightly smaller and larger than the expected fragment. The prominent band approximates a doubling of signal intensity relative to lane 11 (1 copy), which suggests there are probably two copies of the *gus* gene present in these plants consistent with the *bar* blot. The minor bands suggest that a fragment of the *gus* gene exists at different loci within the genome. This interpretation is based on

the size difference and weaker hybridization patterns. The probe did not hybridize to DNA from nontransgenic soybean (lanes 8-9).

In Figure IV. 2.b. the W98 digests (lanes 2-7) show several prominent *gus* hybridization bands, including one at the expected size (5.3 kb). The band of expected size approximates the signal intensity of lane 14 (1 copy). The unexpected bands suggest that a fragment or intact copy of the *gus* gene exists at several different loci within the genome. There appears to be at least 12 different loci where the plasmid, or a plasmid fragment containing the *gus* gene inserted into the genome. This interpretation is based on the number of bands, the size differences between the bands, and the strength of hybridization signals. The probe did not hybridize to DNA from nontransgenic soybean (lanes 8-11).

In Figure IV. 3. the *Xba*1 digests of W62 (lanes 1-5) show a prominent *ampR* hybridization band at the expected size (5.3 kb) and alternate hybridization bands that are smaller than the expected size. The band of expected size approximates a doubling of the signal intensity of lane 9 (1 copy). This suggests there are probably two copies of the amp gene present at this locus in these plants. This is consistent with both the *bar* and *gus* blots. The minor bands suggest that plasmid fragments containing an *ampR* gene exist at different loci within the genome. The smaller sizes of the hybridizing DNA indicate the presence of deletions in the plasmid between the *Xba*1 restriction sites, or the presence of plant genomic DNA/plasmid DNA borders in which the band size is determined by *Xba*1 cleavage in the integrated vector and in the adjacent plant DNA. The probe did not hybridize to DNA from nontransgenic soybean (lanes 6-7).

In Figure IV. 4. the *Xba*1 digests of W98 (lane 1-6) show several prominent *ampR* hybridization bands including one of the expected size (5.3 kb). The expected size band approximates the signal intensity of lane 12 (1 copy). The unexpected bands suggest that a fragment or intact copy of the plasmid containing the *ampR* gene exists at several different loci within the genome. There appears to be at least a total of 12 different loci where the plasmid, or a plasmid fragment containing a portion of the *ampR* gene inserted into the genome. This interpretation is based on the number of bands, the size difference between the bands, and the strength of the hybridization signals. The number of hybridizing loci is consistent with that observed in the *gus* blot. The probe did not hybridize to DNA from nontransgenic soybean (lanes 7-10).

The Figure IV. 5. shows a Southern blot of DNA digested with *BspH*1 and probed with the *ampR* gene (1 kb). The purpose of this digest was to show the presence of intact *ampR* genes by digesting genomic DNA with an enzyme that directly flanks the *ampR* gene in plasmid pWRG2114 (See Figure III. 1.). The digests of W62 (lanes 1-3) and of W98 (lanes 8-11) show a prominent

hybridization band at the expected size (1 kb) and an additional band significantly larger than the expected size. The expected size band for W62 transformants (lanes 1-3) approximates the intensity of lane 6 (1 copy) and supports the 1 to 2 copy number observed in the *Xba*1 Southern blot (Figure IV. 3.). However, the W98 transformants (lanes 8-11) show a very intense signal of the prominent hybridization band at the expected size (1 kb) compared to lane 6 (1 copy). Again, this suggests there are several copies of the *ampR* gene present in these plants. This blot indicates that all but one of the plasmid fragments observed in the *Xba*1 Southern blot (Figure IV. 4.) probably contained an intact *ampR* gene. The unexpected band observed in the upper portion of the blot suggest that at least a fragment of the *ampR* gene exists at a different locus within the genome. The signal intensity of the upper bands suggests that only a single copy of the *ampR* gene exists at this alternate locus. The probe did not hybridize to DNA from nontransgenic soybean (lanes 4 and 7).

In summary, Southern blot analyses show events W62 and W98 contain approximately 2 and 12 intact copies of the *bar*, *gus* and *ampR* genes, respectively. The probes were specific to the introduced sequences in events W62 and W98 since no hybridization was seen with nontransgenic soybean DNA.

<u>Transformation events A2704-12, A2704-21, and A5547-35</u>

In the experiments restriction digested genomic DNA from transgenic plants homozygous for the integrated DNA were run in parallel with a dilution of digested pB2/35AcK vector on an agarose gel. After blotting and hybridization with a *pat*- or an *ampR*- probe the number of copies (intact and partial) of the genes in the soybean genome were quantified by comparing the hybridization intensity of the soybean DNA with the hybridization intensity of the diluted transformation vector. Such reconstruction experiments can only give a rough estimate of the copy number since the parameters for calculation (mass of soybean genome, spectrophotometric quantification of vector and plant DNA, dilution of DNAs, visual comparison of band intensity) are not absolutely precise.

DNA was digested with *Hin*dIII for elucidation of the *pat* gene or Dra1 for elucidation of the *ampR* gene. See Figure III.2. to locate restriction sites in and the probes derived from pB2/35AcK. After separation of the DNA by electrophoresis, the DNA was transferred to a nylon membrane and hybridized with a ^{32}P-labeled *pat* gene fragment (405 bp *Pvu*II fragment) (Figure IV. 6), or with a ^{32}P-labeled *ampR* gene fragment (692 bp *Dra*1 fragment) (Figure IV. 7). Lanes contain 10 ug of restricted DNA. The amount of restricted pB2/35AcK in positive control lanes is equivalent to 0.1 and 1.0 copies of the plasmid integrated in 10 ug of soybean DNA for the *pat* and *ampR* hybridized blots, respectively. The probed membrane was visualized by both autoradiography and by PhosphoImager™ (Molecular Dynamics).

The hybridizing fragments expected and observed when using the *pat* or *ampR* gene as probe are listed in Table IV. 3. The A2704-21 event was obtained following transformation with intact vector DNA. Events A2704-12 and A5547-35 were obtained following digestion of the vector with *PvuI*. This restriction enzyme cleaves the vector DNA at 337 bp and 3456 bp. The former site is upstream of the 5' end of the *35S* gene promoter, while the latter site disrupts the *ampR* coding region.

Table IV. 3. Hybridizing Fragments in Southern Blots of A2704-12, A2704-21, and A5547-35 DNA Probed with the *pat* or *ampR* Gene

Gene Probe and Event	Expected Fragment (kb)[a]	Observed Fragment (kb)
pat - A2704-12	> 4.1	~ 5 bands > 5.0
A2704-21	> 4.1	~ 4 bands > 5.0
A5547-35	> 4.1	< 4.1
ampR - A2704-12	> 0.5	2 bands > 1.0
A2704-21	0.7	3 bands > 1.0
A5547-35	>0.5	none

[a] Expected fragment sizes for 1 copy of inserted vector.

Only the size of the *ampR* gene hybridizing fragment in event A2704-21 can be predicted by the location of restriction enzyme cleavage sites internal to the inserted vector. Those hybridizing fragments whose sizes cannot be predicted result from cleavage in the integrated vector and in the adjacent plant DNA.

In Figure IV. 6. the intensity of the *pat* bands in the A2704-21 (lane 1), A2704-12 (lane 3) and A5547-35 (lane 6) is about 10 fold greater than the signal intensity of the positive control (lane 8, 0.1 copy). This suggests that each hybridizing band in these plants represents the presence of one copy of the *pat* gene. The unexpected number of larger bands suggest that a fragment or intact copy of the *pat* gene exists at several different loci within the genome. The hybridization patterns suggest there are approximately 4, 5 and 1 copies of the *pat* gene present in events A2704-12, A2704-21, and A5547-35, respectively. This interpretation is based on the number and intensity of hybridizing bands. The probe did not hybridize to DNA from nontransgenic soybean (lane 7).

The Figure IV. 7. shows a Southern blot of DNA digested with *Dra*1 and probed with a *Dra*1 *ampR* gene fragment (0.7 kb). The purpose of this digest was two-fold: 1) to indicate the presence of intact *ampR* genes by digesting with an

enzyme that excises about three-quarters of the *ampR* gene in the vector pB2/35SAcK (See Figure III.2.) and, 2) to indicate the number of *ampR* gene fragments present . The digest of A2704-21 (lane 1) shows no hybridization band at the expected size (0.7 kb) for an intact *ampR* gene, but does show three larger bands. The smallest of the three is the least intense, while the largest is the most intense and about 2-fold greater in intensity than the positive control (lane 8, 1 copy). Therefore, there appears to be 4 copies or loci where the plasmid, or a plasmid fragment containing the *ampR* gene inserted into the genome of event A2704-21. Since events A2704-12 (lane 3) and A5547-35 (lane 6) were obtained following transformation with *PvuI* digested vector DNA neither of them are expected to contain an intact *ampR* gene. Event A2704-12 appears to contain 2 loci where a plasmid fragment containing the *ampR* gene is inserted into the genome, whereas, event A5547-35 appears to contain no *ampR* DNA. In conclusion, none of the events A2704-12, A2704-21, and A5547-35 seem to contain an intact *ampR* gene. The probe did not hybridize to DNA from nontransgenic soybean (lanes 7).

In summary, Southern blot analyses show events A2704-12, A2704-21, and A5547-35 contain approximately 4, 5, and 1 intact copies or fragments of the *pat* gene and 4, 2, and 0 fragments of the *ampR* gene, respectively. The probes were specific to the introduced sequences in the events since no hybridization was seen with nontransgenic soybean DNA.

2. Stability of Insertions

To confirm that the integrated DNA in events W62 and W98 remains intact in subsequent generations, the hybridization pattern of progeny from backcrosses to transformation events W62 and W98 was examined. These analyses are shown on the same autoradiographs as the copy number and insert integrity (Figures IV. 1-5). The autoradiographs of the blots show that the integration pattern is unchanged for the number of generations observed (8 for W62.; 10 for W98), thus demonstrating stability of the inserted sequence copies and traits.

We will refer to the *ampR* blots of Xba1 digested W62 (Figure IV. 3) and W98 (Figure IV. 4) DNA to demonstrate insert stability. In Figure IV.3. the same hybridization pattern is found for event W62 R2 generation (lane 1), W62 R3 generation (lane 2), W62 R7 generation (lane 3), [line A x W62 R2] F3 generation (lane 4), and [line B x W62 R2] F3 generation (lane 5). In Figure IV.4. the same hybridization pattern is found for event W98 R2 generation (lane 1), W98 R3 generation (lane 2), W98 R7 generation (lane 3), [line C x W98 R7] F5 generation (lane 4), [line D x W98 R7] F5 generation (lane 5), and [line E x W98 R3] F5 generation (lane 6). The same transformant DNA appears in the other blots, with the exception of the *ampR* blot of *BspH1* digested W62 and W98 DNA where both W62 and W98 R2 and R3 generations are absent (Figure IV. 5). The consistent pattern of hybridization across lines within W62 and within W98 indicates genetic stability of the sequences and traits.

C. Gene Expression in Glufosinate Resistant Soybean

The levels of PAT and GUS proteins in the GRS transformation events W62 and W98 were determined in plant matrices by activity assays. Two different activity assays were performed. The Thin Layer Chromatography (TLC) assay is both a qualitative and quantitative assay that shows whether active PAT enzyme is present. With the ß-glucuronidase flourometric assay the activity of the GUS enzyme can be quantified. To determine whether any of the copies of the *ampR* gene were expressed we performed enzyme activity assays. These analyses show that the *ampR* gene is not expressed in any of the GRS events.

1. PAT and GUS Expression.

PAT activity assays were performed on crude protein extracts of soybean fodder (mature whole plant), and mature seed from plants grown in the field in 1993. The extracts were added to a reaction mix containing ^{14}C-PPT and acetyl-CoA. PAT catalyzes the conversion of L-PPT to N-acetyl-L-PPT in the presence of acetyl-CoA as a co-substrate. Any activity detected in this reaction mix is due to PAT activity since the substrate is not acetylated by other acetyltransferases. Following incubation the reactions were stopped and analyzed by TLC. In TLC formation of ^{14}C-N-acetyl-L-PPT is visualized by autoradiography and quantified by scanning the TLC plate using a scanner equipped with a ^{14}C detector. GUS activity assays were also performed on crude protein extracts of soybean fodder, and mature seed from plants grown in the field in 1993. Activity was detected using the method of Jefferson et al. (1987). Table IV.4. shows the detected PAT and GUS activity. The PAT and GUS activity was determined only for W62 and W98.

PAT activity was detected in all tested samples from transformation events W62 and W98. Activity was higher in fodder and seed from event W62 than from event W98. The data indicate that the majority of the PAT activity was in the seed, since activity in fodder, which contains mature seed, is 3 to 20 fold lower. It appears as though there may be an inverse correlation between copy number of the inserted vector pWRG2114 and level of PAT activity, however, this is not conclusive due to the small sample size.

GUS activity was detected in all event W62 samples, but was not detected in all W98 samples. There was no detected GUS activity in any W98 sample from Illinois. As was seen for PAT activity, when GUS activity was present it was higher in fodder and seed from event W62 than from event W98. Again, the majority of the GUS activity was in the seed, and there may be an inverse correlation between copy number of the inserted vector and the level of GUS activity.

Table IV. 4. Quantities of PAT and GUS in Soybean Matrices

Matrix	Site & Year[a]	Plant Designation[b,c]	ug PAT/ g Matrix[d]	ug GUS/ g Matrix[d]
fodder	AR93	W62	10.8 (6.3-15.3)	56.9 (40.6-68.0)
	IA93	W98	0.75 (0.65-1.0)	0.05 (nd-0.15)
	IL93	W98	10.9 (9.1-12.7)	nd
seed	AR93	W62	217.0 (147.1-267.3)	283.2 (426.9-148.1)
	IA93	W98	27.1 (15.0-39.2)	0.29 (nd-1.74)
	IL93	W98	38.3 (23.5-60.9)	nd

[a] AR = Arkansas; IA = Iowa; IL = Illinois.
[b] Transformed plants were progeny of transformation events W62 and W98.
[c] All plants were treated with GA.
[d] Two to five replicates from fodder samples were analyzed in duplicate; and two to five samples of seed were analyzed. The average values and range for each sample are reported.
[e] nd = no activity detected.

2. *ampR* Expression.

The GRS from transformation events contain zero, two or more intact and/or disrupted copies of the bacterial *ampR* gene (see Section IV.B.1). This gene is under the control of bacterial expression signals and should only be expressed in bacteria. The β-lactamase enzyme confers resistance to β-lactam antibiotics (penicillin, ampicillin, etc.). Although no expression of the *ampR* genes was expected in the transformation events, β-lactamase assays (Tables IV. 5 and 6) were performed to verify that no active protein is generated in GRS.

To detect β-lactamase activity soybean seed extracts from transformed and nontransformed plants were incubated with benzyl-penicillin G. The reaction was stopped by the addition of neocuprione-copper reagent. In this method any penicilloic acid produced by β-lactamase activity is reacted with copper sulfate in the presence of neocuprione to yield a colored complex which has an absorption maximum at 454.5 nm (Cohenford et al., 1988). A unit of activity was quantified in a given volume providing a minimum limit of detection if 0.5 mU/ml. Standard curves were linear when generated using penicillinase standards (0-20 mU) with and without the addition of nontransgenic soybean seed extract (Figure IV.8). Standard reactions containing soybean protein gave a background elevation equivalent to 1-1.7 mU β-lactamase activity. Similar background β-lactamase activity was detected in all nontransformed and transformed plants after the 20

minute reaction time (Tables IV. 5 and 6.). The sensitivity and linear range of the assay were affected little by the addition of seed extract. This assay indicated that there may be an endogenous level of β-lactamase activity of approximately 1.5 mU per 250 ug soluble soybean seed extract (6 mU/gram). Alternatively, the background could be the result of absorbance of 454nm light by seed extract and/or the interaction of the copper reagent with seed extract. From this experiment it is concluded that the inserted *ampR* gene fragments in GRS transformation events produce no functional β-lactamase.

Table IV. 5. β-Lactamase Specific Activity in W62 and W98 Soybean Seeds

Plant[a, b]	β-Lactamase Specific Activity (mU/250 ug soluble seed extract) [c]
A5403 - NT	1.531
A3322 - NT	1.613
A2506 - NT	1.603
A3237 - NT	1.419
A4238 - NT	1.531
W62 R7	1.644
W62 R2 x line A, F3	1.501
W62 R2 x line B, F3	1.185
W98 R7	1.501
W98 R7 x line C, F5	1.532
W98 R7 x line D, F5	1.623
W98 R3 x line E, F5	1.531

a NT = nontransformed.
b Transformed plants were progeny of transformation events W62 and W98.
c One unit (U) of enzyme activity corresponds to 1 uMol/minute at 25 °C and pH 7.0.

Table IV. 6. β-Lactamase Specific Activity in A2704-12, A2704-21 and A5547-35 Soybean Seeds

Plant[a, b]	β-Lactamase Specific Activity (mU/250 ug soluble seed extract) [c]
A2704 - NT	1.220
A2704 - NT	1.092
A2704-12 R1	1.125
A2704-21 R1	0.688
A5547-35 R1	1.092

a NT = nontransformed.
b Transformed plants were progeny of transformation events A2704-12, A2704-21, and A5547-35.
c One unit (U) of enzyme activity corresponds to 1 uMol/minute at 25 °C and pH 7.0.

Figure IV.1. Southern blot of events W62 and W98 hybridized with the *bar* gene . Panel A - DNA was isolated from a R2 generation of event W62 (Lane 1); R3 generation of event W62 (Lane 2); R7 generation of event W62 (Lane 3); F3 generation [line B x W62 R2] (Lane 4); F3 generation [line A x W62 R2] (Lane 5); nontransformed parent A5403 (Lane 6); nontransformed line B (Lane 7); 0.2 copies of pWRG2114 (Lane 8); and 1.0 copies of pWRG2114 (Lane 9). Panel B - DNA was isolated from a R2 generation of event W98 (Lane 2); R3 generation of event W98 (Lane 3); R7 generation of event W98 (Lane 4); F5 generation [line C x W98 R7] (Lane 5); F5 generation [line D x W98 R7] (Lane 6); F5 generation [line E x W98 R3] (Lane 7); nontransformed parent A3322 (Lane 8); nontransformed line F (Lane 9); nontransformed line G (Lane 10); nontransformed line H (Lane 11); 0.2 copies of pWRG2114 (Lane 13); and 1.0 copies of pWRG2114 (Lane 14). DNAs (10 ug) were digested with Xba1. The *bar* gene (442 bp PCR fragment) was used as probe. Panel A, lanes 11-12, and Panel B, lanes 1 and 12, are blank lanes.

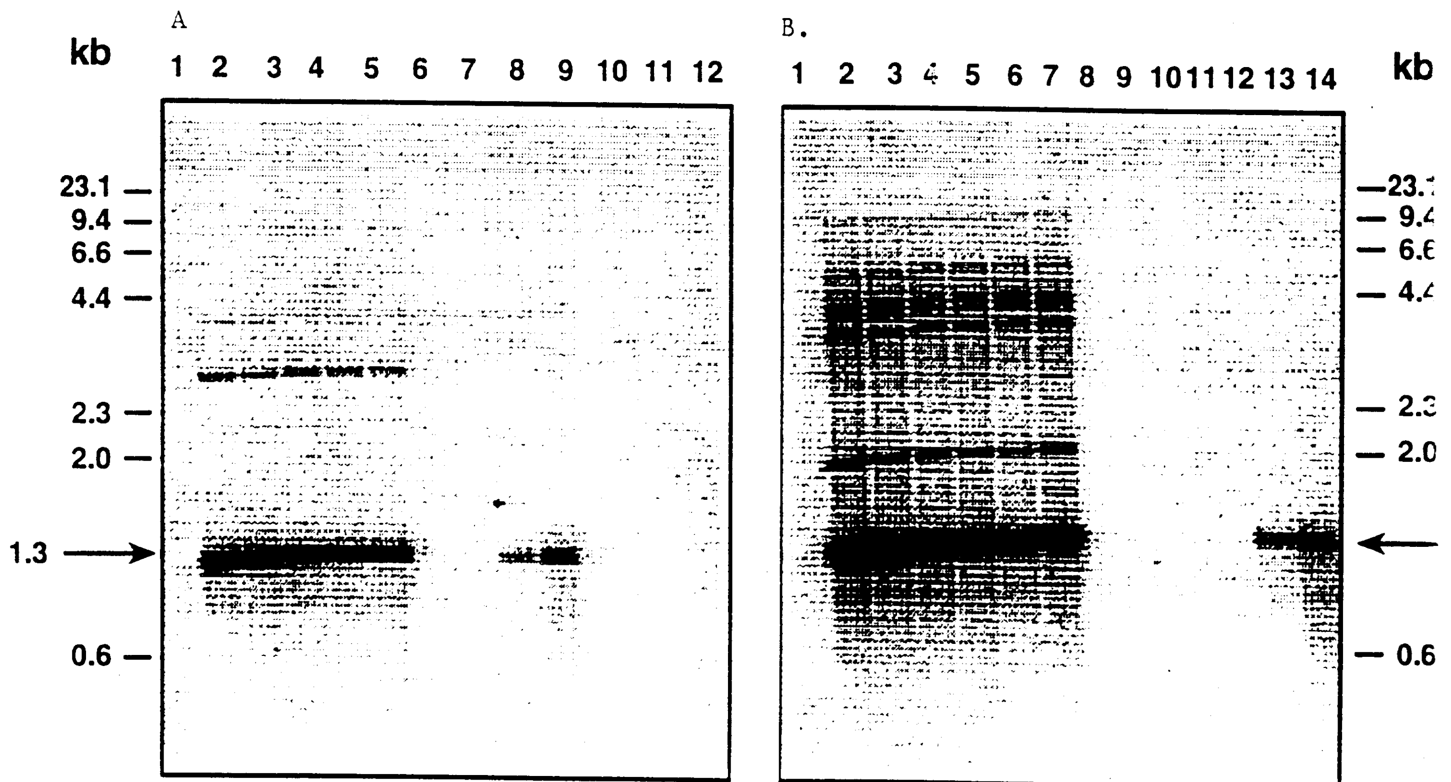


Figure IV.2. Southern blot of events W62 and W98 hybridized with the *gus* gene . Panel A - DNA was isolated from a R2 generation of event W62 (Lane 3); R3 generation of event W62 (Lane 4); R7 generation of event W62 (Lane 5); F3 generation [line B x W62 R2] (Lane 6); F3 generation [line A x W62 R2] (Lane 7); nontransformed parent A5403 (Lane 8); nontransformed line B (Lane 9); 0.2 copies of pWRG2114 (Lane 10); and 1.0 copies of pWRG2114 (Lane 11). Panel B - DNA was isolated from a R2 generation of event W98 (Lane 2); R3 generation of event W98 (Lane 3); R7 generation of event W98 (Lane 4); F5 generation [line C x W98 R7] (Lane 5); F5 generation [line D x W98 R7] (Lane 6); F5 generation [line E x W98 R3] (Lane 7); nontransformed parent A3322 (Lane 8); nontransformed line F (Lane 9); nontransformed line G (Lane 10); nontransformed line H (Lane 11); 0.2 copies of pWRG2114 (Lane 13); and 1.0 copies of pWRG2114 (Lane 14). DNAs (10 ug) were digested with *Xba*1. The *gus* gene (1880 bp *Nco*1/*Sma*1 fragment) was used as probe. Panel A, lanes 1-2 and 13-14; and Panel B, lanes 1 and 12, are blank or molecular weight marker lanes.

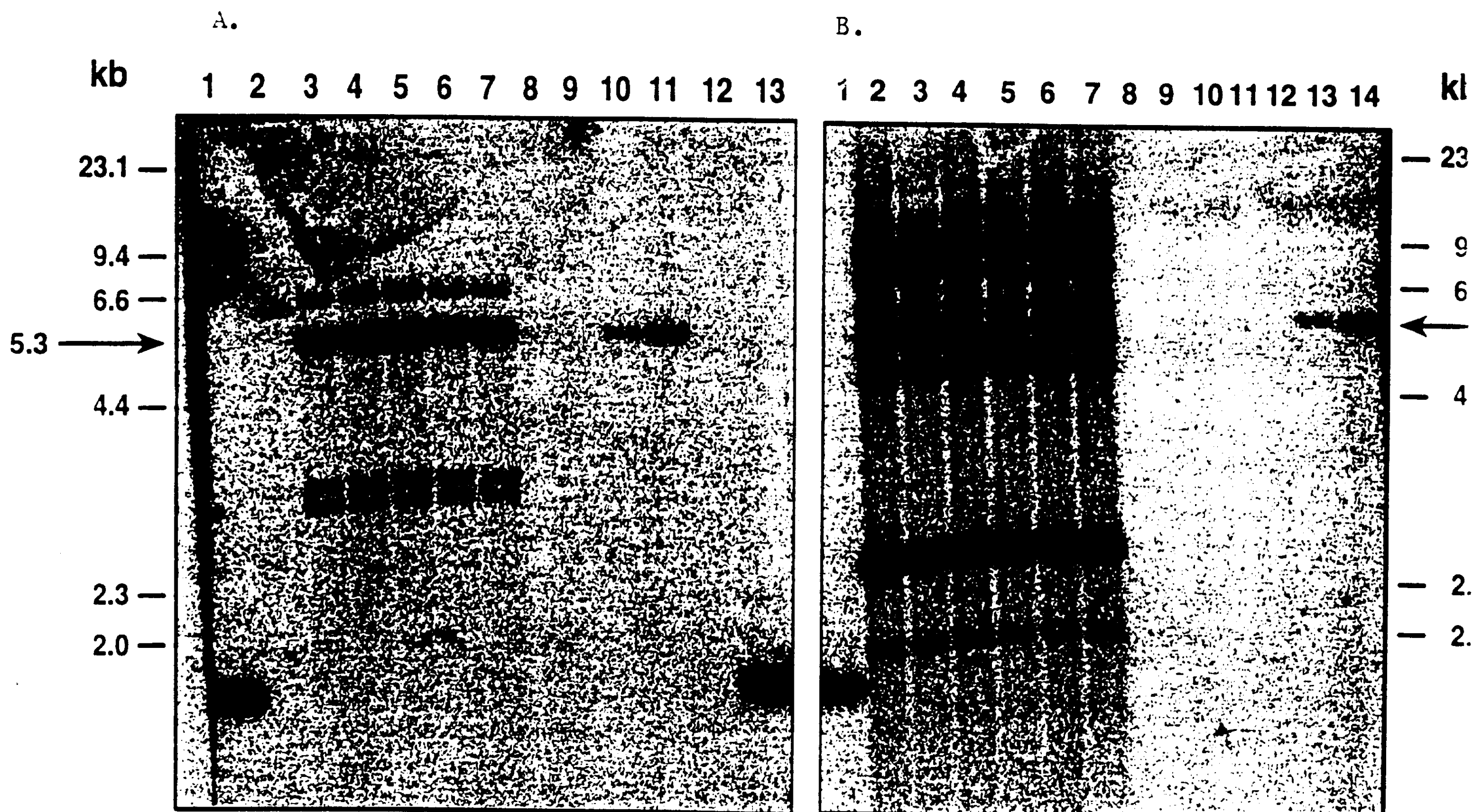


Figure IV.3. Southern blot of Xba1 digests of event W62 hybridized with the *ampR* gene. DNA was isolated from a R2 generation of event W62 (Lane 1); R3 generation of event W62 (Lane 2); R7 generation of event W62 (Lane 3); F3 generation [line B x W62 R2] (Lane 4); F3 generation [line A x W62 R2] (Lane 5); nontransformed parent A5403 (Lane 6); nontransformed line B (Lane 7); 0.2 copies of pWRG2114 (Lane 8); and 1.0 copies of pWRG2114 (Lane 9). DNAs (10 ug) were digested with *Xba*1. The *ampR* gene (1008 bp *BspH*1 fragment) was used as probe.

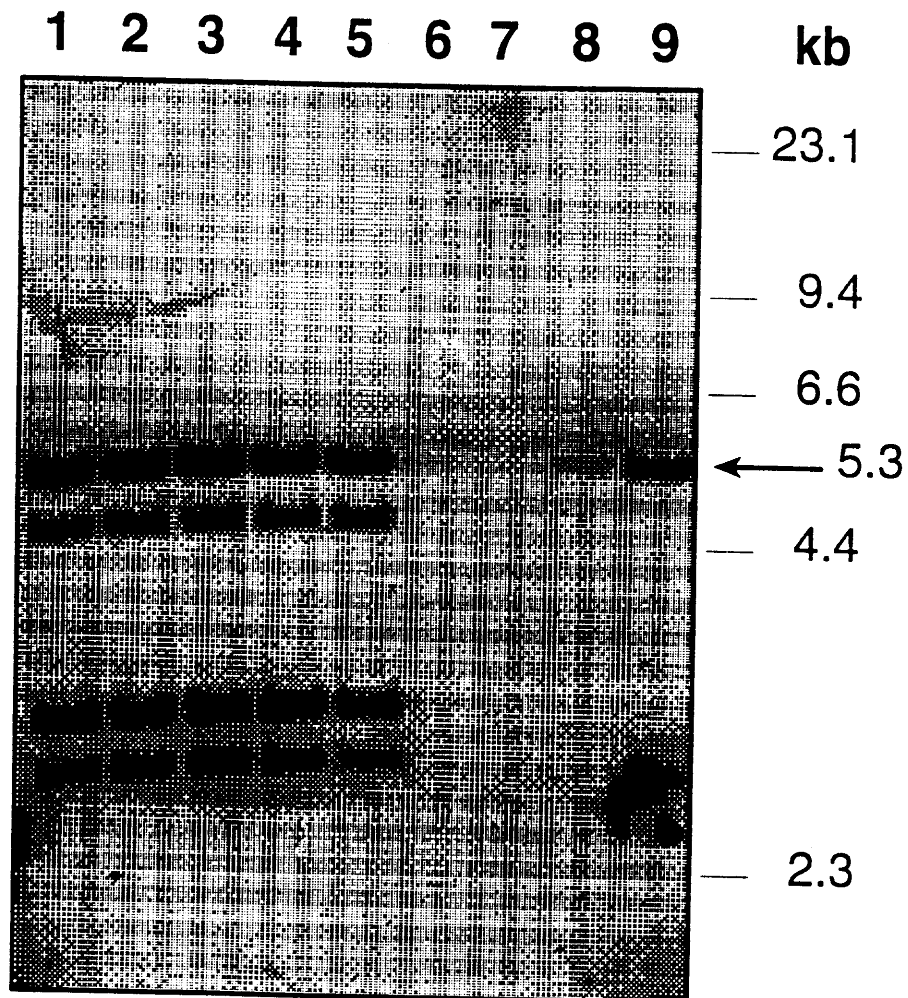


Figure IV.4. Southern blot of Xba1 digests of event W98 hybridized with the *ampR* gene. DNA was isolated from a R2 generation of event W98 (Lane 1); R3 generation of event W98 (Lane 2); R7 generation of event W98 (Lane 3); F5 generation [line C x W98 R7] (Lane 4); F5 generation [line D x W98 R7] (Lane 5); F5 generation [line E x W98 R3] (Lane 6); nontransformed parent A3322 (Lane 7); nontransformed line F (Lane 8); nontransformed line G (Lane 9); nontransformed line H (Lane 10); 0.2 copies of pWRG2114 (Lane 11); and 1.0 copies of pWRG2114 (Lane 12). DNAs (10 ug) were digested with *Xba*1. The *ampR* gene (1008 bp *BspH*1 fragment) was used as probe.

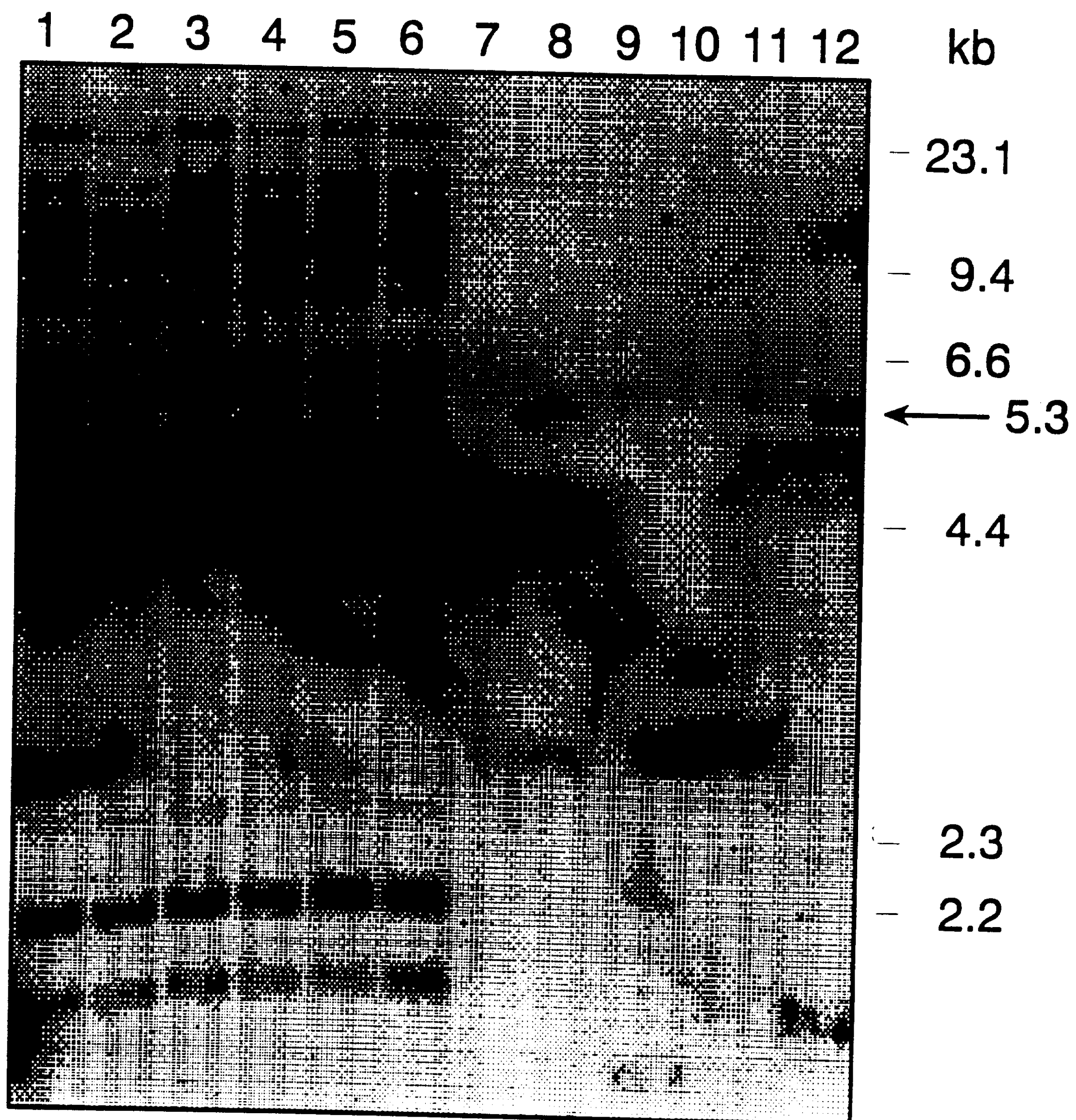


Figure IV.5. Southern blot of BspH1 digests of events W62 and W98 hybridized with the *ampR* gene. DNA was isolated from a R7 generation of event W62 (Lane 1); F3 generation [line B x W62 R2] (Lane 2); F3 generation [line A x W62 R2] (Lane 3); nontransformed parent A5403 (Lane 4); 0.2 copies of pWRG2114 (Lane 5); and 1.0 copies of pWRG2114 (Lane 6); nontransformed parent A3322 (Lane 7); R7 generation of event W98 (Lane 8); F5 generation [line C x W98 R7] (Lane 9); F5 generation [line D x W98 R7] (Lane 10); and F5 generation [line E x W98 R3] (Lane 11). DNAs (10 ug) were digested with *BspH*1. The *ampR* gene (1008 bp *BspH*1 fragment) was used as probe.

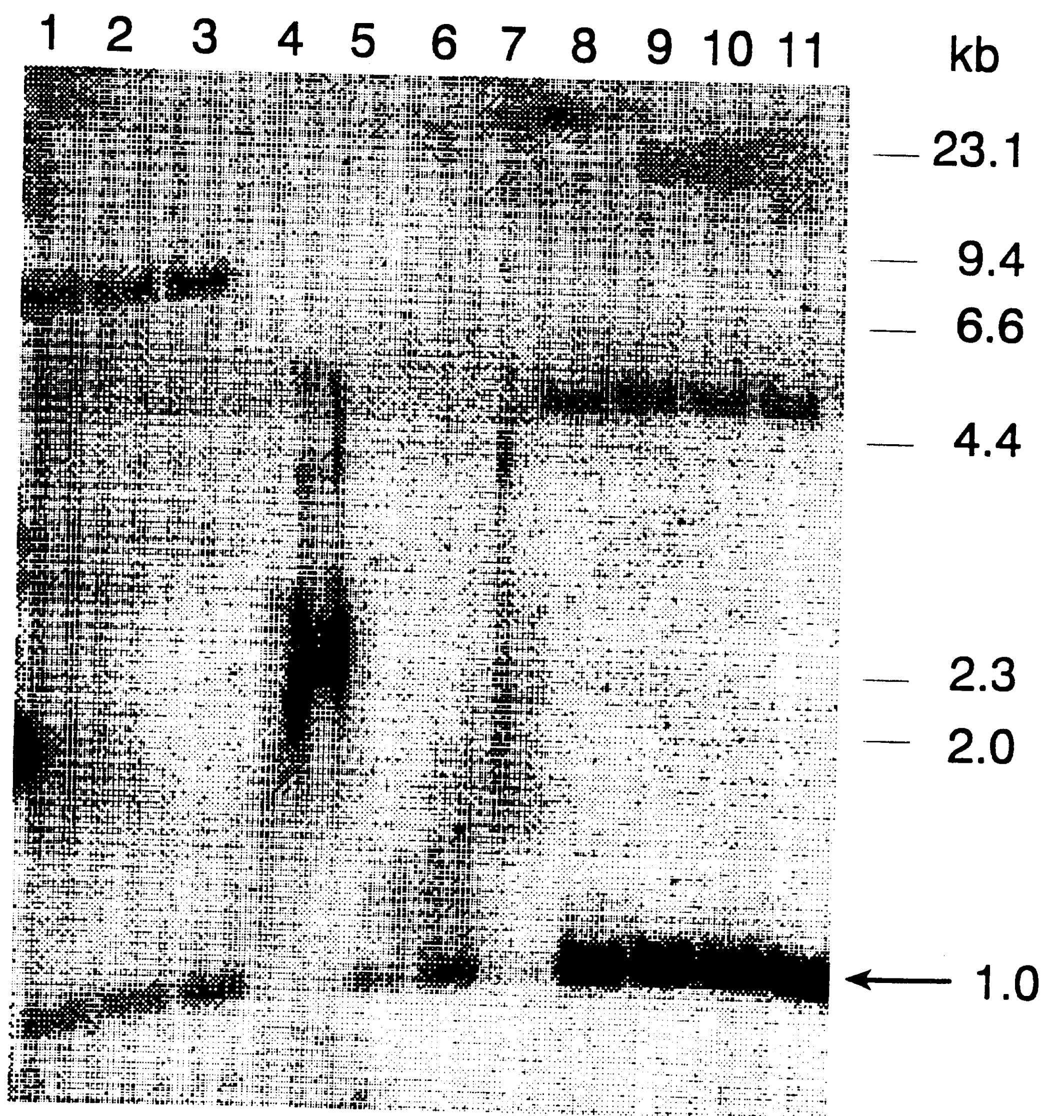


Figure IV.6. Southern blot of events A2704-12, A2704-21, and A5547-35 hybridized with the *pat* gene. DNA was isolated from a R1 generation of event A2704-21 (lane 1); R1 generation of event A2704-12 (lane 3); R1 generation of event A5547-35 (lane 6); nontransformed soybean (lane 7); and 0.1 copies of pB2/35SAcK. DNA's (10 ug) were digested with HindIII. The *pat* gene (405 bp *Pvu*II fragment) was used as probe. Lanes 2 and 4 contain DNA from events not considered in this document.

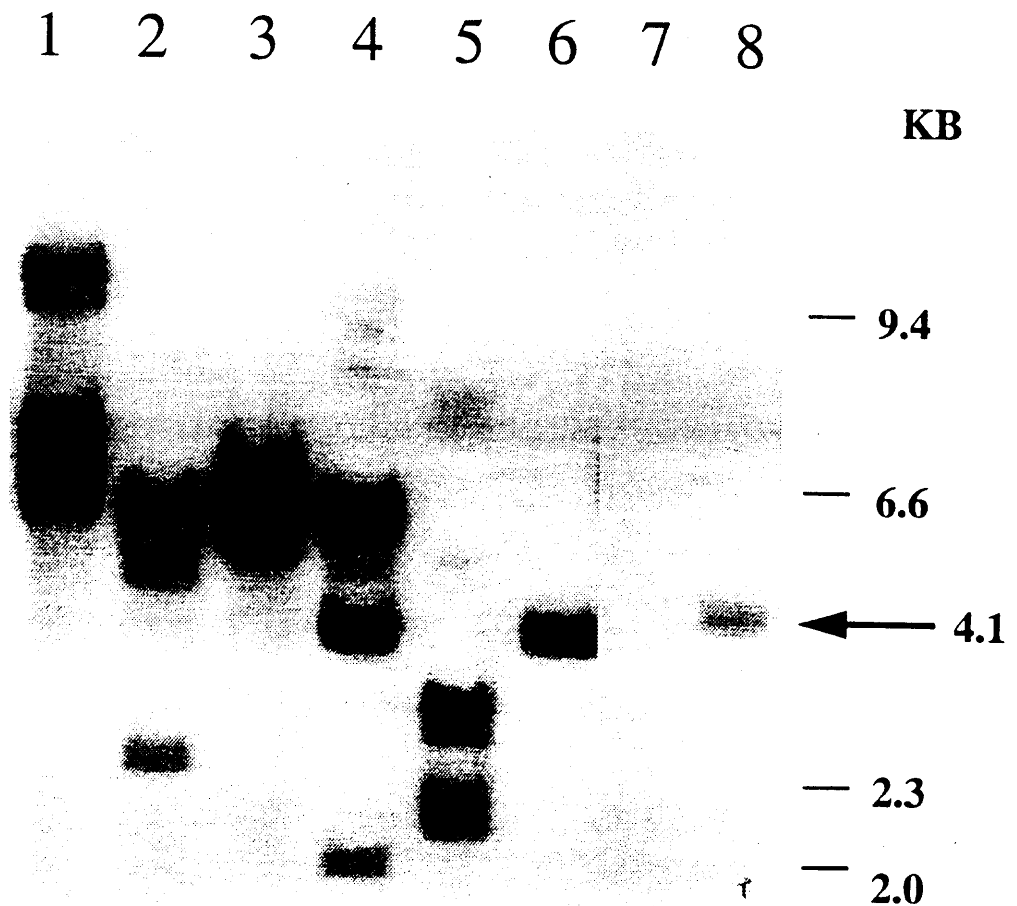


Figure IV.7. Southern blot of events A2704-12, A2704-21, and A5547-35 hybridized with the *ampR* gene. DNA was isolated from a R1 generation of event A2704-21 (lane 1); R1 generation of event A2704-12 (lane 3); R1 generation of event A5547-35 (lane 6); nontransformed soybean (lane 7); and 0.1 copies of pB2/35SAcK. DNA's (10 ug) were digested with Dra1. The *ampR* gene (692 bp Dra1 fragment) was used as probe. Lanes 2 and 4 contain DNA from events not considered in this document.

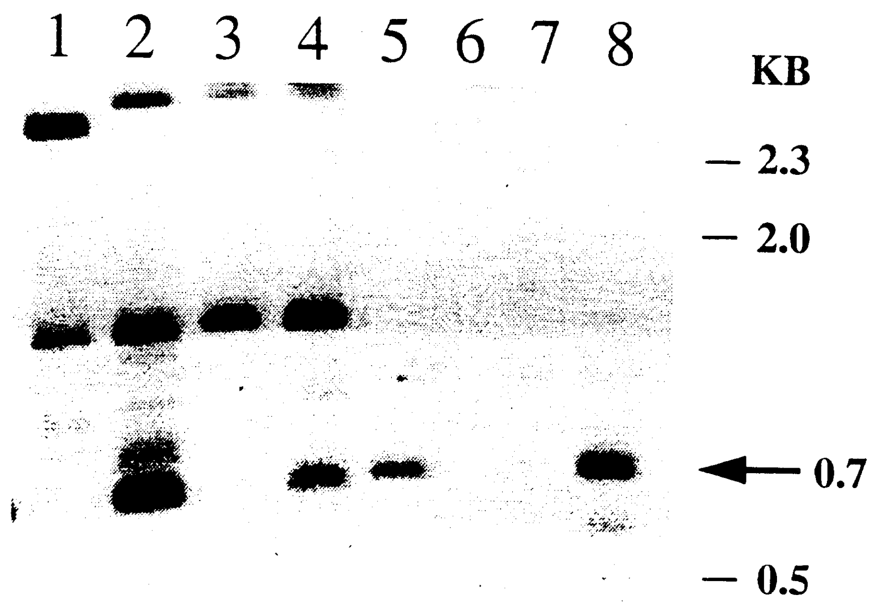
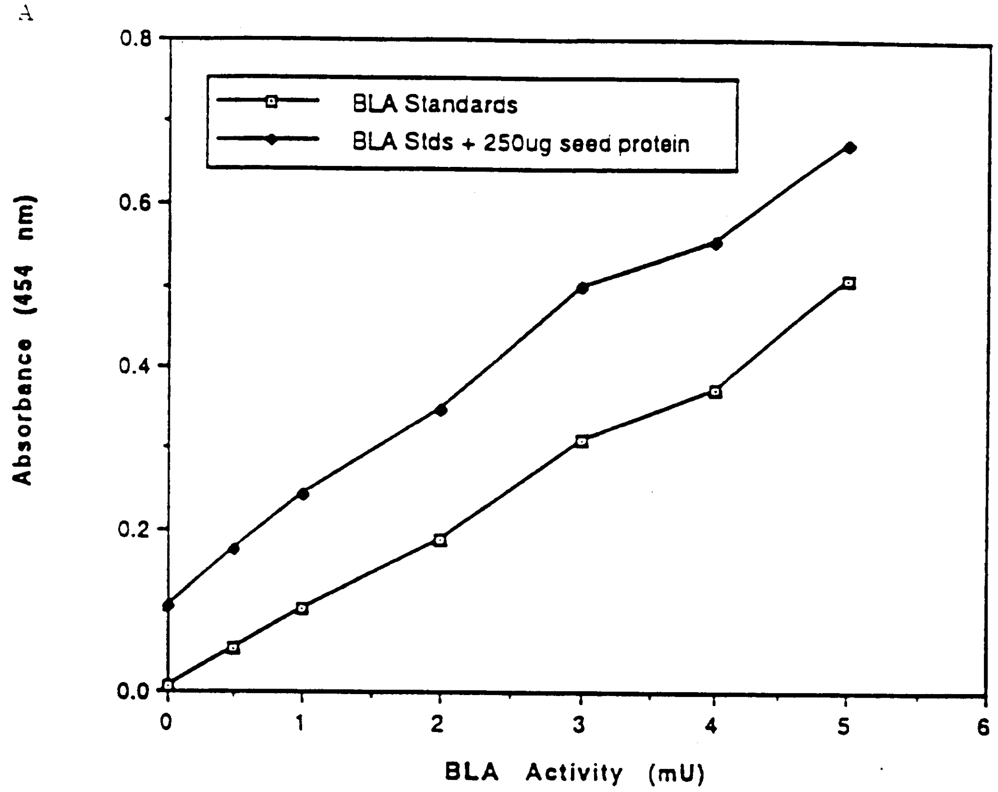
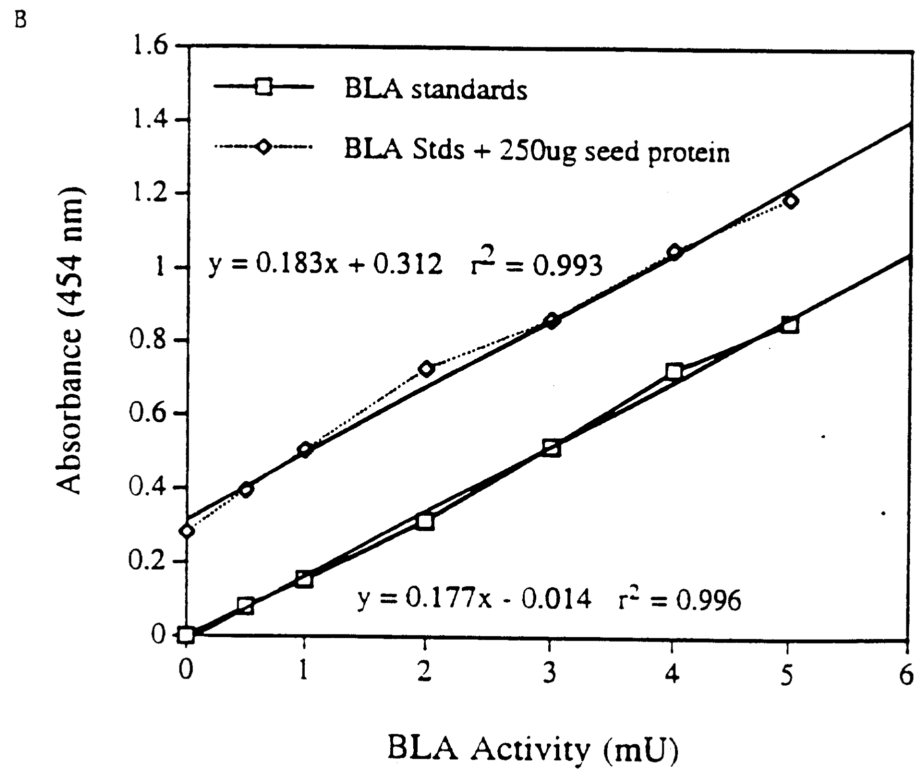


Figure IV.8. ß-Lactamase standard curves for events. Panel A - Standard curves prepared for events W62 and W98. Panel B - Standard curves prepared for events A2704-12, A2704-21, and A5547-35. Standards were prepared as described in Section IV.C.2. BLA = ß-Lactamase.

A



B



V. Agronomic Performance of Glufosinate Resistant Soybean

As was seen for events W62 and W98, there were no differences in morphology, and in disease or insect resistance between the events and nontransgenic counterparts. In addition, the expected segregation ratios were observed for a single dominant *pat* locus (See termination report 95-122-03 in Appendix 2). In these trials, when sprayed with the herbicide, all plants exhibited a high level of glufosinate resistance, indicating that the gene is stably integrated and expressed.

A. Field Tests of Events W62 and W98

Transformation events W62 and W98 have been field tested by AgrEvo USA Company since 1992 in the primary soybean growing regions of the United States. These tests have occurred at approximately 195 sites under field release authorizations granted by APHIS (USDA authorizations: permits 90-274-05, 91-051-03, 91-203-01, 92-043-02, 92-043-03, 93-090-01, 93-047-02, 93-047-03; notifications 93-120-31, 93-120-35, 93-127-02, 93-270-03, 94-080-03, 94-090-02, 94-131-01, 95-034-02, 95-069-01, 95-069-02, 95-069-03, 95-069-04, 95-069-05, 95-069-06, 95-069-07, 95-069-08, 95-069-09, 95-069-10, 95-069-11, 95-069-12, 95-079-02, 95-115-04, 95-135-04, 95-142-02. Transformation events W62 and/or W98 have also been field tested in Canada, where test results have been similar to those in the United States. Transformation events A2704-12, A2704-21, and A5547-35 were evaluated in the field in 1995 at 2 sites under authorizations granted by APHIS (95-034-02 and 95-122-03).

The great majority of the trials in the United States have been efficacy trials in which the plants have been sprayed with different rates of GA to determine the level of weed control and soybean resistance. However, observations were also made on agronomic characteristics and disease and pest characteristics. Appendix 2 contains termination reports submitted to the USDA for the environmental releases that have been completed in the United States.

B. Agronomic Characteristics

Company researchers, university cooperators, and soybean breeders made visual observations of many agronomic traits of GRS events including plant morphology, time of flowering, stand count, plant height, crop injury due to chemical application, root lodging, stalk lodging and yield. For all traits evaluated a nontransgenic genetic counterpart was also evaluated. Qualitative evaluations and certain quantitative evaluations were made during the 1992 through 1995 growing seasons. For all agronomic information gathered, there were no differences between transformation events and the nontransgenic counterparts, with the single exception that the nontransgenic material was not resistant to GA application (See termination reports in Appendix 2). A more

thorough discussion of overwintering ability, germination and stand count for events W62 and W98 is made below.

Although overwintering and germination of GRS events W62 and W98 were not directly tested under field conditions, stand counts were made upon emergence of the plants in the spring, and sites were monitored for volunteers in subsequent seasons. Plots have been observed for volunteers after the 1992/93,1993/94 and 1994/95 winter months and no volunteers were ever observed. At the beginning of growing seasons evaluation of seedling emergence was made throughout the US on hundreds of progeny of transformation events W62 and W98 in up to 15 backgrounds. In all cases field emergence was observed to be similar in transgenic and nontransgenic soybeans.

Comparisons were also made to determine the possibility of reduced yield for transformation events W62 and W98. The comparisons were made throughout the US on hundreds of progeny of transformation events W62 and W98 and nontransgenic hybrids in up to 15 backgrounds. Comparisons between spray rates showed no significance between a one time application of no (0X rate), 500 (1X), or 2000 (4x rate) gm ai/hectare of GA. AgrEvo prefers that finished lines be resistant to up to 1500 gm ai/ hectare of GA even though we are pursuing registration of GA on GRS with a 1 or 2 time application at a rate of 400 gm ai/hectare of GA. Qualitative observation of yield has not identified any reduction when 2 applications of 400 gm ai/hectare of GA are applied to transformation events W62 and W98 material.

C. Disease and Pest Characteristics

There are many viral, bacterial, fungal, nematode, and insect pests that can damage soybean and cause disease (Athow, 1987; Riggs and Schmitt, 1987; Ross, 1987; Turnipseed and Kogan, 1987). In any given year one such pest infestation could result in severe damage and yield reduction to the soybean crop. However, high disease pressure is rare in soybean. Company researchers and cooperators made visual observations for plant pathogenic organisms in trials containing GRS events W62 and W98 during the 1992, 1993, 1994 and 1995 growing seasons. Visual observations in trials containing GRS events A2704-12, A2704-21, and A5547-35 were made during the 1995 growing season. Such observations revealed some minor pathogen infections but no infestations (see Appendix 2). Diseases observed included brown stem rot (*Cephalosporium gregatum)*, Phytophthora root rot (*Phytophthora megasperma* f. sp. *glycinea*), downey mildew (*Peronospora trifoliorum*), powdery mildew (*Microaphaera diffusa*), stem canker (*Diaporthe*), Sclerotinia stem rot (white mold) (*Sclerotinia sclerotiorum*), and soybean cyst nematode (*Heterodera glycines*). Insect pest infestations of green cloverworm (*Plathypena scabra*), bean leaf beetle (*Cerotoma trifurcata*), soybean looper (*Pseudoplusia includens*),

stink bugs (*Acrosternum hilare, Euschistus servus, Nezara viridula)*, leafhoppers (*Empoasca* species), Japanese beetle (*Popilliae japonica)*, twospotted spider mite (*Tetranychus urticae*), whiteflies (*Bemisia* spp.), clover leaf weevil (*Hypera punctata*), and chinch bug (*Blissus leucopterus leucopterus*) were observed at release sites. Whenever pests were observed there was no differences in damage or populations found between GRS events and nontransgenic counterparts. In addition, no differences were observed between plots of GRS events W62 abd W98 treated with no, 400, and 1500 gm GA/hectare (Appendix 2). The integration of vector DNA did not affect the inherent resistance of the parent cultivar to either Phytophthora root rot or soybean cyst nematode (See Section III.B.; and termination reports 93-043-03, 93-047-02, and 94-090-02 in Appendix 2). GRS events did not influence susceptibility to disease or pest organisms in diverse genetic backgrounds and environments.

VI. Potential for Environmental Impact from Noncontained Use of Glufosinate Resistant Soybeans

A. The Herbicide Glufosinate-ammonium and Current Uses

Ammonium-DL-homoalanin-4-yl-(methyl) phosphinate (glufosinate-ammonium, GA) is a non-selective, non-systemic herbicide that controls a broad spectrum of annual and perennial grass and broadleaf weeds. The L-form (L-PPT) is the active component of GA. This form is also the active portion of the naturally occurring antibiotic bialaphos (see Section III.D.). It has a similar structure and shape as glutamic acid, and is therefore called a glutamic acid analog. It inhibits the enzyme GS which converts glutamic acid and ammonia into glutamine (Leason et al., 1982). L-PPT's ability to bind irreversibly with glutamic acid results in the buildup of ammonia that inhibits photophosphorylation in photosynthesis (Wild 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.

GA must be absorbed through the green plant tissues 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. GA is rapidly degraded in the soil by microorganisms, not only in well aerated soils, but also in soils with stagnant moisture (Hoechst Technical Bulletin, 1991). Therefore, GA has very low residual soil activity and does not injure seedlings before emergence. Both GA 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, GA does not affect soil microflora or alter the number or mass of earthworm populations (Hoechst Technical Bulletin, 1991). Application of GA 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, GA is not a hazard to birds or mammals. There were no changes in tumor incidence after lifetime exposure to GA in rats and mice. No mutagenic activity was detected in a battery of mutagenicity tests. When administered to pregnant animals, GA produced no adverse effects on fetuses at doses which were not toxic to the mothers (USEPA, 1995).

There are presently no registered uses for GA in soybean. However, GA is registered for use as a non-selective herbicide on turf (tradename Finale®), for light industrial use (tradename Liberty™ NC), and on apples, grapes, and tree nuts (tradename Rely®) in the United States. Outside the United States, GA is registered for use on plantation crops, tree nuts, and vines, and for industrial/non-agricultural weed control under a variety of tradenames including

Basta® and Ignite®. It is registered for selective use on canola in Canada (Liberty®)

B. Effects on Agricultural and Cultivation Practices of Soybean

1. Current Practices

In the United States soybeans are grown in the midwestern and southern states. About 78% of the soybeans are grown in twelve midwestern states. Soybeans are grown in rotation primarily with corn in the midwest; and corn, wheat, or cotton in the southern US. Volunteer soybeans are generally not a problem in rotational crops. Seed does not normally germinate after being in the field over the winter. Many herbicides commonly used in rotational crops, such as those containing triazines [atrazine, cyanazine], phenoxys [2, 4-D], or benzoic acids [dicamba] cause injury to soybeans.

Nearly all the soybean acreage in the United States is treated with a herbicide. Products are applied, pre-plant, pre-emergence and post-emergence to the soybean crop. Herbicide programs in soybeans can vary due to the geographic area, weed spectrum and tillage practices. Farmers have traditionally made 2-3 applications per year, and depending on the row width of plantings and tillage, 1-2 additional treatments could be made. A preventative broadcast treatment of pre-plant and pre-emergence products for grass control is still the predominate primary treatment today. Over one-half of the acres in the midwest receive a trifluralin [Treflan], pendimethalin [Prowl], metolachlor [Dual] or clomazone [Command] treatment. In the midwest, and on traditional grown soybeans a second application for the control of broadleaf weeds is usually made post-emergence. A third spot application for escapes of grasses or broadleaf weeds is sometimes made. Volunteer corn escapes can receive another treatment with a post-emergence grass product.

Soybeans growing in the southern US might demand an additional treatment for escapes because of the length of the growing seasons. Over 60% of the soybean receive a pre-plant incorporated or a pre-emergence product for initial control of grasses. One or two broadleaf applications can be made depending on the season. A third application for the control of johnsongrass (*Sorghum halepense*) and other perennial weeds is usually made.

Throughout the soybean belt the use of post emergence grass or broadleaf control products has increased over the past decade. These products have increased with the increase of narrow row, or solid seeded soybean plantings, and with the increase usage of no-till systems. These products were initially used for escapes from the pre-plant or pre-emergence products. Post-emergence products used in a program approach have gained a significant

portion of the market today. The main reason for increase use of post-emergence products is their improved selectivity, such as bentazon [Basagran] for broadleaf and sethoxydin [Poast] or fluazifop [Fusilade] for grasses.

The increased use of no-till systems has also lead to the increase use of burndown, or non-selective products. This additional treatment can be made before, during, or after planting, but before emergence of the crop. The use of pre-emergence products along with the burndown product is common. A third or fourth treatment of problem weeds usually follows.

Problem weeds in soybean include velvetleaf (*Abulilon theophrasti*), morningglory (*Ipomea* spp.), shattercane (*Sorghum bicolor*), johnsongrass (*Sorghum halepense*), quackgrass (*Agropyron repens*), fall panicum (*Panicum dichotomiflorum*), foxtails (*Setaria* spp.), wild proso millet (*Panicum miliaceum*), woolly cupgrass (*Eriochloa villosa*) and sicklepod (*Cassia obtusifolia*). Pigweeds (*Amaranthus* spp.), ragweeds (*Ambrosia* spp.), common lambsquarters (*Chenopodium album*), and smartweeds (*Polygonum* spp.) are broadleaf concerns that can cause problems if not properly managed. Perennial species such as hemp dogbane (*Apocynum cannabinum*) and Canada thistle (*Cirsium arvense*) are difficult to control.

Soybean Weed Control Programs

1. Normal Midwest Program - following corn
    - Fall tillage, chisel or disk
    - Spring tillage before planting
    - Apply PPI or pre-emergence of trifluralin [Treflan] or pendimethalin [Prowl] for residual grass control
    - Apply post-emergence broadleaf product; bentazon [Basagran] or aciflurofen [Blazer]
    - Cultivate once or twice
    - Apply grass post-emergence product sethoxydin [Poast] or fluazifop [Fusilade] for escapes

2. No-Till System
    - Apply burndown (gramoxone [Paraquot] or glyphosate [Roundup])
    - Apply pre-emergence soil residual herbicides, grass and broadleaf
    - Apply post-emergence grass and broadleaf combinations (separate applications may be required due to weed stage of growth)
    - Apply post-emergence product for control of perennial weeds, i.e., johnsongrass.

3. Low Input Program
   - Fall tillage
   - Spring disk
   - Apply pre-plant incorporated product
   - Plant
   - Apply post-emergence product as needed
   - Cultivate two times

2. <u>Possible Effect of Glufosinate Resistant Soybean on Current Practices</u>

The use of GA will have no effect on the normal growth patterns of GRS plants. No effect on agronomic traits of GRS will be seen. Positive effects in soybean cultivation will come from changes in tillage practices and herbicide use patterns. The broad spectrum, post-emergence activity of GA will help increase the amount of conservation and no-till acres of soybean planted in the United States. The use of GA together with GRS will increase the adoption of post-emergence chemistry. Growers have the desire for a broad spectrum, post-emergence herbicide, and an opportunity to move away from pre-emergence and residually active compounds. GRS and GA may positively impact current agronomic practices in soybean by 1) offering a new broad spectrum, post-emergence weed control system; 2) providing the opportunity to continue to move away from pre-emergence and residually active compounds; 3) providing a novel chemistry and mode of action that provides very effective weed resistance management in soybean acreage; 4) offering the use of an environmentally sound and naturally occurring herbicide; 5) encouraging herbicide use on an as needed basis; 6) offering a wide window of application giving farmers confidence that they can move to post-emergence control without assuming more risk; and 7) allowing the application of less total pounds of active ingredient than used presently.

3. <u>Likelihood of Appearance of Glufosinate-resistant Weeds</u>

The only foreseeable way by which a weed could develop true resistance to GA is through sexual transmission of the *pat* gene. This can and will occur where the crop and the related wild species are growing together and can exchange genetic material and produce fertile progeny . However, for soybean in the United States sexual transfer to weed relatives does not occur (see Section II).

Today there are large numbers of herbicide resistant weed biotypes, with over half of them resistant to triazines (Powles and Holtum, 1994). GA is unrelated to triazines and has a different mode of action, i.e., it inhibits GS. It is unlikely that weeds or any plant species will spontaneously develop resistance to GA under selective pressure, because a plant must either develop mutant forms of GS that do not bind L-PPT, but still recognize glutamic acid, and/or evolve a L-PPT

detoxification system. Experimental work to create GA resistant crop plants by selection has been ongoing for several years with no success. Below is an accounting of attempts to create GA resistant crop plants in the laboratory by selection for mutants that can tolerate L-PPT or overproduce GS.

Over the last 10 years AgrEvo has not succeeded in selecting a glufosinate resistant soybean mutant from protoplast cultures. There have been no survivors when wildtype soybean protoplasts are placed on medium containing L-PPT. On the other hand, using sulfonylureas as selective agents we have been able to select 44 independent sulfonylurea resistant mutants within 3 months. Using fenoxaprop-ethyl as a selective agent we have been able to select 2 independent fenoxaprop resistant mutants during one year. In all cases, there is a correlation with observations of resistant weed populations, i.e., glufosinate resistant weeds have never been observed, but weeds resistant to the other chemicals have been found.

Glutamine synthetase exists in multiple isozymic forms in different plant organs (McNally et al., 1983). These forms can be cytosol or plastid localized, and encoded by a multigene family. Overproduction of the GS isozymes could provide a degree of tolerance to L-PPT. Donn et al. (1984) selected alfalfa suspension cell lines that were more tolerant to L-PPT than wild-type cells. These cell lines have a 3- to 7- fold increase in their GS activity, due to an increase in GS mRNA resulting from amplification of a GS gene. When the amplified GS gene, under the regulation of the CaMV 35S promoter, was integrated into the tobacco plant genome, a 5-fold increase in GS specific activity and a 20-fold increase in tolerance to L-PPT was measured *in vitro* (Eckes et al., 1989). Neither the amino acid composition of the plant tissue was altered significantly by GS overproduction; nor were the fertility and growth of the overproducing GS plants affected. Although overproduction of GS in plants has been demonstrated following intensive laboratory manipulation, it is doubtful that weeds will be selected in nature which overproduce GS, thereby conferring commercial levels of resistance to GA.

The likelihood that GS mutants will occur that do not bind L-PPT, but still recognize glutamic acid seems to be extremely low. *In vitro* mutagenesis studies in Dr. Howard Goodman's lab, Massachusetts General Hospital, several years ago showed that GS mutants that could no longer bind L-PPT could be obtained for the alfalfa GS gene (personal communication, Günter Donn, AgrEvo GmbH). However, these mutants were very ineffective in using glutamic acid as a substrate. A plant bearing such a mutation would have difficulties surviving because its ability to detoxify ammonia would be seriously decreased. This theoretical consideration is in accordance with the observations *in vitro* and in the field.

In conclusion, the likelihood of appearance of glufosinate-resistant weeds in the United States is extremely low to none.

C. Effects on Glufosinate Resistant Soybean on Non-target Organisms

GRS transformation events W62 and W98 have been field tested at numerous sites across the US since 1992, and other events have been tested recently, and no toxicity or alteration of population levels have been observed for beneficial insects, birds or other species that frequent soybean fields (see termination reports, Appendix 2). There were no qualitative differences between beneficial species and populations present on transgenic and nontransgenic soybean plants. This observation was expected since GRS contain a gene which encodes a protein that is naturally occurring (see Section III. D. 4. and Section VI.A.), and this protein shares no homology with proteins that are known to be toxic (see Section VI. E.).

D. Weediness Potential of Glufosinate Resistant Soybean

Soybean is generally not regarded as a weed. Its inherent agronomic characteristics, such as higher yield, resistance to pod shattering, plant height and resistance to lodging, have been intensified because soybean is naturally a self-fertilized plant. Soybeans, of the subgenus *Soja*, to which cultivated soybeans belong, are annuals reproducing only by seed. The seed does not remain viable for long periods of time. Collectively, these characteristics have reduced cultivated soybean chances of becoming a weed even though it has been intensively grown for many years over a wide region of the United States (USDA-APHIS, 1994; Rissler and Mellon, 1993). The USDA -APHIS (1994) has stated that cultivated soybean "is an annual crop and is considered to be highly domesticated, well characterized crop plant that is not persistent in undisturbed environments without human intervention." No reference on weed species lists the cultigen *G. max* or *G. gracilis* as a weed (USDA-APHIS, 1994). *Glycine soja*, however, is listed as a common weed in Japan by Holm et al. (1979), but is not listed in other texts of weeds found in Japan (Kasahara, 1982; Nemoto, 1982). Soybean is not listed as a noxious weed in the United States (USDA-AMS, 1994).

The introduction of resistance to the herbicide GA has not caused GRS to become a weed. GRS soybean retains the same growth rate and growth habit as nontransgenic soybean (see Appendix 2, and Section V.B). It continues to be an annual with uniform germination. In addition, GRS is equally susceptible to disease and insect pests as its nontransgenic counterparts (Section V.C. and Appendix 2). GRS volunteer soybeans were not observed (see Appendix 2). If one chooses to eliminate GRS transformation events, and their progeny by chemical management, they can be removed by treatment with herbicides other than GA (termination report 94-080-03, Appendix 2). Trials where GRS was treated with glyphosate or dicamba demonstrate that introduction of the PAT enzyme does not impart cross tolerance to chemicals with modes of action that differs from GA.

E. Indirect Effects of Glufosinate Resistant Soybean on other Agricultural Products

There are three major markets for soybean products: beans, oil and meal. Raw soybeans are generally not consumed by livestock and humans, but is subjected to a number of processing steps including high temperature drying, toasting and oil extraction (temperatures up to 114°C [235°F]. AgrEvo GmbH (Schulz, 1993a) has conducted studies on purified, synthetic PAT enzyme which show that the enzyme is both heat and acid labile. The enzyme loses 100% of its activity upon incubation at 75°C (167°F) or greater for 30 minutes. At pH values of 4 or less it is inactive after exposure for 30 minutes. The heat treatments used for the processing of soybeans should eliminate most PAT activity. To confirm this AgrEvo USA Company has processed soybeans, however, these studies are not yet complete.

Should there be any PAT enzyme remaining after these treatments, the only route of exposure for humans and livestock to PAT in GRS soybean would be via oral ingestion. In addition, animals could be exposed orally to PAT present in unprocessed soybeans. AgrEvo GmbH has confirmed experimentally that PAT protein and *pat* DNA in a plant matrix is rapidly degraded *in vitro* by the gastric juices from swine, chicken, and cattle (Schneider, 1993; Schulz 1993b). These animals represent the three primary types of gastric systems among livestock. It has also been experimentally confirmed that PAT is readily degraded in simulated human gastric fluids within minutes (Schulz, 1994).

The PAT enzyme does not have the characteristics of an allergen or a toxin. It is acid and heat labile and contains no glycosylation motifs (Eckes, 1994). The protein has no homology to proteins other than PAT genes from other organisms (Eckes, 1994). The substrate specificity for the PAT enzyme is very strict in that the only substrate is L-PPT. Neither any protein amino acid nor D-PPT is acetylated by PAT (Schulz 1993a). Acetyl transferases are abundant and ubiquitous in nature where they share the common function of transferring an acetyl group from acetyl CoA to a substrate. Acetyl transferases differ in substrates and the metabolic pathways in which they function (Webb, 1992).

Based on 1) the substrate specificity of PAT; 2) the physicochemical properties of PAT; 3) its rapid degradation upon ingestion; 4) the low levels of PAT in whole tissues (Table IV.2.); and 5) the ubiquitous presence of acetyl transferases in nature, no adverse effects are predicted if the PAT enzyme is a minor constituent of human and animal food.

F. Potential for Gene Transfer to Other Organisms

1. Outcrossing with wild species

As discussed in Section II, no wild relatives of soybean can be found within the continental United States, although some wild perennials are found in the South Pacific territories of the United States (USDA-APHIS, 1994; Rissler and Mellon, 1993). There are no known reports of natural hybridization between cultivated soybean and its wild perennials (Singh and Hymowitz, 1985; Newell and Hymowitz, 1978; USDA-APHIS, 1994). Accordingly, there is little probability of unaided crosses between GRS transformation events and wild relatives in the United States, and little potential for loss of biodiversity among wild relatives in the United States.

2. Outcrossing to cultivated soybean

Self-fertilization is the primary method of pollination in soybean. In fact, Certified Seed Regulations (7 CFR 201.76-201.78) acknowledge the low possibility of cross pollination in the safeguards set up for Foundation, Registered and Certified seed. The most stringent regulations exist in the production of Foundation seed, where blocks of soybeans of different lines can be grown adjacent to one another, as long as the distance is sufficient to prevent contamination by mechanical (not pollination) mixing. Unless chance outcrossing occurs to other cultivated soybeans, the *bar* and *pat* loci will be maintained in the germplasm just like any other trait. Although GRS or its progeny from commercial soybean production may arise as volunteers the following season, volunteers can be eliminated by the application of herbicides other than GA (See Section VI.D.).

3. Transfer of genetic information to organisms with which it cannot interbreed

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. Indeed, as described earlier in this document, the *bar* gene present in GRS events W62 and W98 and the synthetic *pat* gene present in the events A2704-12, A2704-21 and A5547-35 are derived

from genes isolated from a naturally occurring soil microbe. Transfer of an *ampR* gene fragment from GRS events also would not pose a risk, since β-lactamase genes are common in microbes in nature.

VII. Statement of Grounds Unfavorable

No unfavorable information and data has been demonstrated for GRS Transformation Events W62 and W98, or the events A2704-12, A2704-21 and A5547-35.

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IX. Appendices

Appendix 1. DNA Sequence Data

Appendix 2. USDA Field Trial Termination Reports

Appendix 3. Literature Reprints

96-068-01p

Appendix 1. DNA Sequence Data

pWRG2114 Sequence
Tuesday, May 9, 1995 10:49 AM

Sequence Range: 1 to 6656

```
         10         20         30         40         50         60
    *    *    *    *    *    *    *    *    *    *    *    *
AGACGAAAGG GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT AATAATGGTT

         70         80         90        100        110        120
    *    *    *    *    *    *    *    *    *    *    *    *
TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT

                       >P3,_beta-Lactamase_promoter
                                  |
        130        140        150 |      160        170        180
    *    *    *    *    *    *    *    *    *    *    *    *
TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA

        190        200        210        220        230        240
    *    *    *    *    *    *    *    *    *    *    *    *
TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT
                      __________a___BETA-LACTAMASE____a__________>

        250        260        270        280        290        300
    *    *    *    *    *    *    *    *    *    *    *    *
TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT
__________a__________a___BETA-LACTAMASE____a__________a__________>

        310        320        330        340        350        360
    *    *    *    *    *    *    *    *    *    *    *    *
GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG
__________a__________a___BETA-LACTAMASE____a__________a__________>

        370        380        390        400        410        420
    *    *    *    *    *    *    *    *    *    *    *    *
ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG
__________a__________a___BETA-LACTAMASE____a__________a__________>

        430        440        450        460        470        480
    *    *    *    *    *    *    *    *    *    *    *    *
CTATGTGGCG CGGTATTATC CCGTGTTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA
__________a__________a___BETA-LACTAMASE____a__________a__________>

        490        500        510        520        530        540
    *    *    *    *    *    *    *    *    *    *    *    *
CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT
__________a__________a___BETA-LACTAMASE____a__________a__________>

        550        560        570        580        590        600
    *    *    *    *    *    *    *    *    *    *    *    *
GGCATGACAG TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC
__________a__________a___BETA-LACTAMASE____a__________a__________>

        610        620        630        640        650        660
    *    *    *    *    *    *    *    *    *    *    *    *
AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT GCACAACATG
__________a__________a___BETA-LACTAMASE____a__________a__________>

        670        680        690        700        710        720
    *    *    *    *    *    *    *    *    *    *    *    *
GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC
__________a__________a___BETA-LACTAMASE____a__________a__________>

        730        740        750        760        770        780
```

```
       *       *       *       *       *       *       *       *       *       *       *       *
GACGAGCGTG ACACCACGAT GCCTGTAGCA ATGGCAACAA CGTTGCGCAA ACTATTAACT
__________a__________a___BETA-LACTAMASE____a__________a__________>

       790        800        810        820        830        840
       *       *       *       *       *       *       *       *       *       *       *       *
GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA
__________a__________a___BETA-LACTAMASE____a__________a__________>

       850        860        870        880        890        900
       *       *       *       *       *       *       *       *       *       *       *       *
GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT
__________a__________a___BETA-LACTAMASE____a__________a__________>

       910        920        930        940        950        960
       *       *       *       *       *       *       *       *       *       *       *       *
GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC
__________a__________a___BETA-LACTAMASE____a__________a__________>

       970        980        990       1000       1010       1020
       *       *       *       *       *       *       *       *       *       *       *       *
TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA
__________a__________a___BETA-LACTAMASE____a__________a__________>

      1030       1040       1050       1060       1070       1080
       *       *       *       *       *       *       *       *       *       *       *       *
CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTGGT AACTGTCAGA CCAAGTTTAC
__________a____BETA-LACTAMASE___a__________a__>

      1090       1100       1110       1120       1130       1140
       *       *       *       *       *       *       *       *       *       *       *       *
TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG

      1150       1160       1170       1180       1190       1200
       *       *       *       *       *       *       *       *       *       *       *       *
ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG

      1210       1220       1230       1240       1250       1260
       *       *       *       *       *       *       *       *       *       *       *       *
TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC

      1270       1280       1290       1300       1310       1320
       *       *       *       *       *       *       *       *       *       *       *       *
TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG

      1330       1340       1350       1360       1370       1380
       *       *       *       *       *       *       *       *       *       *       *       *
CTACCAACTC TTTTTCCGAA GGTAACTGGC TTCAGCAGAG CGCAGATACC AAATACTGTC

      1390       1400       1410       1420       1430       1440
       *       *       *       *       *       *       *       *       *       *       *       *
CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC

      1450       1460       1470       1480       1490       1500
       *       *       *       *       *       *       *       *       *       *       *       *
CTCGCTCTGC TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC

      1510       1520       1530       1540       1550       1560
       *       *       *       *       *       *       *       *       *       *       *       *
GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG AACGGGGGGT

      1570       1580       1590       1600       1610       1620
```

```
      *         *         *         *         *         *         *         *         *         *         *         *
TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT

      1630      1640      1650      1660      1670      1680
      *    *    *    *    *    *    *    *    *    *    *    *
GAGCATTGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC

      1690      1700      1710      1720      1730      1740
      *    *    *    *    *    *    *    *    *    *    *    *
GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT

      1750      1760      1770      1780      1790      1800
      *    *    *    *    *    *    *    *    *    *    *    *
TATAGTCCTG TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA

                <Origin_of_replication
                     |
      1810      1820 |    1830      1840      1850      1860
      *    *    *    *    *    *    *    *    *    *    *    *
GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGGGCCTT TTTACGGTTC CTGGCCTTTT

      1870      1880      1890      1900      1910      1920
      *    *    *    *    *    *    *    *    *    *    *    *
GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA

      1930      1940      1950      1960      1970      1980
      *    *    *    *    *    *    *    *    *    *    *    *
TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT

      1990      2000      2010      2020      2030      2040
      *    *    *    *    *    *    *    *    *    *    *    *
CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC

      2050      2060      2070      2080      2090      2100
      *    *    *    *    *    *    *    *    *    *    *    *
CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA

      2110      2120      2130      2140      2150      2160
      *    *    *    *    *    *    *    *    *    *    *    *
ACGCAATTAA TGTGAGTTAG CTCACTCATT AGGCACCCCA GGCTTTACAC TTTATGCTTC

      2170      2180      2190      2200      2210      2220
      *    *    *    *    *    *    *    *    *    *    *    *
CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG

      2230      2240      2250      2260      2270      2280
      *    *    *    *    *    *    *    *    *    *    *    *
ACCATGATTA CGCCAAGCTT GCATGCCTGC AGGTCGAGGA ACATGGTGGA GCACGACACT

      2290      2300      2310      2320      2330      2340
      *    *    *    *    *    *    *    *    *    *    *    *
CTCGTCTACT CCAAGAATAT CAAAGATACA GTCTCAGAAG ACCAAAGGGC TATTGAGACT

      2350      2360      2370      2380      2390      2400
      *    *    *    *    *    *    *    *    *    *    *    *
TTTCAACAAA GGGTAATATC GGGAAACCTC CTCGGATTCC ATTGCCCAGC TATCTGTCAC

      2410      2420      2430      2440      2450      2460
      *    *    *    *    *    *    *    *    *    *    *    *
TTCATCAAAA GGACAGTAGA AAAGGAAGGT GGCACCTACA AATGCCATCA TTGCGATAAA

      2470      2480      2490      2500      2510      2520
```

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      *       *       *       *       *       *       *       *       *       *       *       *
GGAAAGGCTA TCGTTCAAGA TGCCTCTGCC GACAGTGGTC CCAAAGATGG ACCCCCACCC

        2530        2540        2550        2560        2570        2580
      *       *       *       *       *       *       *       *       *       *       *       *
ACGAGGAGCA TCGTGGAAAA AGAAGACGTT CCAACCACGT CTTCAAAGCA AGTGGATTGA

        2590        2600        2610        2620        2630        2640
      *       *       *       *       *       *       *       *       *       *       *       *
TGTGATATCT CCACTGACGT AAGGGATGAC GCACAATCCC ACTATCCTTC GCAAGACCCT

                                          >Alfalfa_Mosaic_Virus_Leader
                                                     |
        2650        2660        2670        2680     |  2690        2700
      *       *       *       *       *       *       *       *       *       *       *       *
TCCTCTATAT AAGGAAGTTC ATTTCATTTG GAGAGGACAC CAAGCTTTTT ATTTTTAATT

        2710        2720        2730        2740        2750        2760
      *       *       *       *       *       *       *       *       *       *       *       *
TTCTTTCAAA TACTTCCACC ATGGTCTTAC GTCCTGTAGA AACCCCAACC CGTGAAATCA
                      __________b_BETA-GLUCURONIDASE__b__________>

        2770        2780        2790        2800        2810        2820
      *       *       *       *       *       *       *       *       *       *       *       *
AAAAACTCGA CGGCCTGTGG GCATTCAGTC TGGATCGCGA AAACTGTGGA ATTGATCAGC
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

        2830        2840        2850        2860        2870        2880
      *       *       *       *       *       *       *       *       *       *       *       *
GTTGGTGGGA AAGCGCGTTA CAAGAAAGCC GGGCAATTGC TGTGCCAGGC AGTTTTAACG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

        2890        2900        2910        2920        2930        2940
      *       *       *       *       *       *       *       *       *       *       *       *
ATCAGTTCGC CGATGCAGAT ATTCGTAATT ATGCGGGCAA CGTCTGGTAT CAGCGCGAAG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

        2950        2960        2970        2980        2990        3000
      *       *       *       *       *       *       *       *       *       *       *       *
TCTTTATACC GAAAGGTTGG GCAGGCCAGC GTATCGTGCT GCGTTTCGAT GCGGTCACTC
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

        3010        3020        3030        3040        3050        3060
      *       *       *       *       *       *       *       *       *       *       *       *
ATTACGGCAA AGTGTGGGTC AATAATCAGG AAGTGATGGA GCATCAGGGC GGCTATACGC
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

        3070        3080        3090        3100        3110        3120
      *       *       *       *       *       *       *       *       *       *       *       *
CATTTGAAGC CGATGTCACG CCGTATGTTA TTGCCGGGAA AAGTGTACGT ATCACCGTTT
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

        3130        3140        3150        3160        3170        3180
      *       *       *       *       *       *       *       *       *       *       *       *
GTGTGAACAA CGAACTGAAC TGGCAGACTA TCCCGCCGGG AATGGTGATT ACCGACGAAA
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

        3190        3200        3210        3220        3230        3240
      *       *       *       *       *       *       *       *       *       *       *       *
ACGGCAAGAA AAAGCAGTCT TACTTCCATG ATTTCTTTAA CTATGCCGGA ATCCATCGCA
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>
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            3250       3260       3270       3280       3290       3300
          *    *     *    *     *    *     *    *     *    *     *    *
GCGTAATGCT CTACACCACG CCGAACACCT GGGTGGACGA TATCACCGTG GTGACGCATG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3310       3320       3330       3340       3350       3360
          *    *     *    *     *    *     *    *     *    *     *    *
TCGCGCAAGA CTGTAACCAC GCGTCTGTTG ACTGGCAGGT GGTGGCCAAT GGTGATGTCA
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3370       3380       3390       3400       3410       3420
          *    *     *    *     *    *     *    *     *    *     *    *
GCGTTGAACT GCGTGATGCG GATCAACAGG TGGTTGCAAC TGGACAAGGC ACTAGCGGGA
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3430       3440       3450       3460       3470       3480
          *    *     *    *     *    *     *    *     *    *     *    *
CTTTGCAAGT GGTGAATCCG CACCTCTGGC AACCGGGTGA AGGTTATCTC TATGAACTGT
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3490       3500       3510       3520       3530       3540
          *    *     *    *     *    *     *    *     *    *     *    *
GCGTCACAGC CAAAAGCCAG ACAGAGTGTG ATATCTACCC GCTTCGCGTC GGCATCCGGT
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3550       3560       3570       3580       3590       3600
          *    *     *    *     *    *     *    *     *    *     *    *
CAGTGGCAGT GAAGGGCGAA CAGTTCCTGA TTAACCACAA ACCGTTCTAC TTTACTGGCT
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3610       3620       3630       3640       3650       3660
          *    *     *    *     *    *     *    *     *    *     *    *
TTGGTCGTCA TGAAGATGCG GACTTACGTG GCAAAGGATT CGATAACGTG CTGATGGTGC
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3670       3680       3690       3700       3710       3720
          *    *     *    *     *    *     *    *     *    *     *    *
ACGACCACGC ATTAATGGAC TGGATTGGGG CCAACTCCTA CCGTACCTCG CATTACCCTT
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3730       3740       3750       3760       3770       3780
          *    *     *    *     *    *     *    *     *    *     *    *
ACGCTGAAGA GATGCTCGAC TGGGCAGATG AACATGGCAT CGTGGTGATT GATGAAACTG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3790       3800       3810       3820       3830       3840
          *    *     *    *     *    *     *    *     *    *     *    *
CTGCTGTCGG CTTTAACCTC TCTTTAGGCA TTGGTTTCGA AGCGGGCAAC AAGCCGAAAG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3850       3860       3870       3880       3890       3900
          *    *     *    *     *    *     *    *     *    *     *    *
AACTGTACAG CGAAGAGGCA GTCAACGGGG AAACTCAGCA AGCGCACTTA CAGGCGATTA
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3910       3920       3930       3940       3950       3960
          *    *     *    *     *    *     *    *     *    *     *    *
AAGAGCTGAT AGCGCGTGAC AAAAACCACC CAAGCGTGGT GATGTGGAGT ATTGCCAACG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

            3970       3980       3990       4000       4010       4020
          *    *     *    *     *    *     *    *     *    *     *    *
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AACCGGATAC CCGTCCGCAA GTGCACGGGA ATATTTCGCC ACTGGCGGAA GCAACGCGTA
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4030      4040      4050      4060      4070      4080
   *    *    *    *    *    *    *    *    *    *    *    *
AACTCGACCC GACGCGTCCG ATCACCTGCG TCAATGTAAT GTTCTGCGAC GCTCACACCG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4090      4100      4110      4120      4130      4140
   *    *    *    *    *    *    *    *    *    *    *    *
ATACCATCAG CGATCTCTTT GATGTGCTGT GCCTGAACCG TTATTACGGA TGGTATGTCC
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4150      4160      4170      4180      4190      4200
   *    *    *    *    *    *    *    *    *    *    *    *
AAAGCGGCGA TTTGGAAACG GCAGAGAAGG TACTGGAAAA AGAACTTCTG GCCTGGCAGG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4210      4220      4230      4240      4250      4260
   *    *    *    *    *    *    *    *    *    *    *    *
AGAAACTGCA TCAGCCGATT ATCATCACCG AATACGGCGT GGATACGTTA GCCGGGCTGC
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4270      4280      4290      4300      4310      4320
   *    *    *    *    *    *    *    *    *    *    *    *
ACTCAATGTA CACCGACATG TGGAGTGAAG AGTATCAGTG TGCATGGCTG GATATGTATC
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4330      4340      4350      4360      4370      4380
   *    *    *    *    *    *    *    *    *    *    *    *
ACCGCGTCTT TGATCGCGTC AGCGCCGTCG TCGGTGAACA GGTATGGAAT TTCGCCGATT
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4390      4400      4410      4420      4430      4440
   *    *    *    *    *    *    *    *    *    *    *    *
TTGCGACCTC GCAAGGCATA TTGCGCGTTG GCGGTAACAA GAAAGGGATC TTCACTCGCG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4450      4460      4470      4480      4490      4500
   *    *    *    *    *    *    *    *    *    *    *    *
ACCGCAAACC GAAGTCGGCG GCTTTTCTGC TGCAAAAACG CTGGACTGGC ATGAACTTCG
__________b__________b_BETA-GLUCURONIDASE__b__________b__________>

     4510      4520      4530      4540      4550      4560
   *    *    *    *    *    *    *    *    *    *    *    *
GTGAAAAACC GCAGCAGGGA GGCAAACAAT GAATCAACAA CTCTCCTGGC GCACCATCGT
__________BETA-GLUCURONIDASE_______b__>

     4570      4580      4590      4600      4610      4620
   *    *    *    *    *    *    *    *    *    *    *    *
CGGCTACAGC CTCGGTGGGG AATTCCTGCA GGGATCCCCG GGGATCGTTC AAACATTTGG

     4630      4640      4650      4660      4670      4680
   *    *    *    *    *    *    *    *    *    *    *    *
CAATAAAGTT TCTTAAGATT GAATCCTGTT GCCGGTCTTG CGATGATTAT CATATAATTT

     4690      4700      4710      4720      4730      4740
   *    *    *    *    *    *    *    *    *    *    *    *
CTGTTGAATT ACGTTAAGCA TGTAATAATT AACATGTAAT GCATGACGTT ATTTATGAGA

     4750      4760      4770      4780      4790      4800
   *    *    *    *    *    *    *    *    *    *    *    *
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TGGGTTTTTA TGATTAGAGT CCCGCAATTA TACATTTAAT ACGCGATAGA AAACAAAATA

      4810       4820       4830       4840       4850       4860
    *    *     *    *     *    *     *    *     *    *     *    *
TAGCGCGCAA ACTAGGATAA ATTATCGCGC GCGGTGTCAT CTATGTTACT AGATCCGTCG

      4870       4880       4890       4900       4910       4920
    *    *     *    *     *    *     *    *     *    *     *    *
ACTCTTCTAG AGGATCCGAA TTCCTTTGTT AACCATCATT TAGTGACACA ATTAACTTAA

      4930       4940       4950       4960       4970       4980
    *    *     *    *     *    *     *    *     *    *     *    *
ATTTAGAAGA GACTAATTTC AACCCACAAG ATGGAAATGC AAGGACAATG GGTTCTTGAA

      4990       5000       5010       5020       5030       5040
    *    *     *    *     *    *     *    *     *    *     *    *
GTTATTTGGA AACAAAGCAA CACAACACAA TAGCTTATTC ATTAGTTCNC ATCCATTAAT

      5050       5060       5070       5080       5090       5100
    *    *     *    *     *    *     *    *     *    *     *    *
TTCCATGAAG TCCGAAAACA GAAACCAAAA ATACAGGATG ATGAAATCTC ATAAAGCAAA

      5110       5120       5130       5140       5150       5160
    *    *     *    *     *    *     *    *     *    *     *    *
ACAAATGGGA ATGTAGTTTA AGTACAACAA ACAAAGTGGG GAGGCTACAG ATGCTTTGCA

      5170       5180       5190       5200       5210       5220
    *    *     *    *     *    *     *    *     *    *     *    *
AACTTCNAAT GACTTACGGA TCCCCGGGGA TCCCTGCAGT TACTATCAGA TCTCGGTGAC
                                                   <_____BAR_____

      5230       5240       5250       5260       5270       5280
    *    *     *    *     *    *     *    *     *    *     *    *
GGGCAGGACC GGACGGGGCG GTACCGGCAG GCTGAAGTCC AGCTGCCAGA AACCCACGTC
<_________c__________c________BAR_________c__________c___________

      5290       5300       5310       5320       5330       5340
    *    *     *    *     *    *     *    *     *    *     *    *
ATGCCAGTTC CCGTGCTTGA AGCCGGCCGC CCGCAGCATG CCGCGGGGGG CATATCCGAG
<_________c__________c________BAR_________c__________c___________

      5350       5360       5370       5380       5390       5400
    *    *     *    *     *    *     *    *     *    *     *    *
CGCCTCGTGC ATGCGCACGC TCGGGTCGTT GGGCAGCCCG ATGACAGCGA CCACGCTCTT
<_________c__________c________BAR_________c__________c___________

      5410       5420       5430       5440       5450       5460
    *    *     *    *     *    *     *    *     *    *     *    *
GAAGCCCTGT GCCTCCAGGG ACTTCAGCAG GTGGGTGTAG AGCGTGGAGC CCAGTCCCGT
<_________c__________c________BAR_________c__________c___________

      5470       5480       5490       5500       5510       5520
    *    *     *    *     *    *     *    *     *    *     *    *
CCGCTGGTGG CGGGGGGAGA CGTACACGGT CGACTCGGCC GTCCAGTCGT AGGCGTTGCG
<_________c__________c________BAR_________c__________c___________

      5530       5540       5550       5560       5570       5580
    *    *     *    *     *    *     *    *     *    *     *    *
TGCCTTCCAG GGGCCCGCGT AGGCGATGCC GGCGACCTCG CCGTCCACCT CGGCGACGAG
<_________c__________c________BAR_________c__________c___________

      5590       5600       5610       5620       5630       5640
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     *         *          *         *          *         *          *         *          *         *          *         *
 CCAGGGATAG CGCTCCCGCA GACGGACGAG GTCGTCCGTC CACTCCTGCG GTTCCTGCGG
<__________c__________c_________BAR_________c__________c__________

       5650       5660       5670       5680       5690       5700
     *    *     *    *     *    *     *    *     *    *     *    *
 CTCGGTACGG AAGTTGACCG TGCTTGTCTC GATGTAGTGG TTGACGATGG TGCAGACCGC
<__________c__________c_________BAR_________c__________c__________

       5710       5720       5730       5740       5750       5760
     *    *     *    *     *    *     *    *     *    *     *    *
 CGGCATGTCC GCCTCGGTGG CACGGCGGAT GTCGGCCGGG CGTCGTTCTG GGCTCACCAT
<__________c__________c_________BAR_________c__________c__________

       5770       5780       5790       5800       5810       5820
     *    *     *    *     *    *     *    *     *    *     *    *
 GGTGGAAGTA TTTGAAAGAA AATTAAAAAT AAAAAGCTTG GTGTCCTCTC CAAATGAAAT

       5830       5840       5850       5860       5870       5880
     *    *     *    *     *    *     *    *     *    *     *    *
 GAACTTCCTT ATATAGAGGA AGGGTCTTGC GAAGGATAGT GGGATTGTGC GTCATCCCTT

       5890       5900       5910       5920       5930       5940
     *    *     *    *     *    *     *    *     *    *     *    *
 ACGTCAGTGG AGATATCACA TCAATCCACT TGCTTTGAAG ACGTGGTTGG AACGTCTTCT

       5950       5960       5970       5980       5990       6000
     *    *     *    *     *    *     *    *     *    *     *    *
 TTTTCCACGA TGCTCCTCGT GGGTGGGGGT CCATCTTTGG GACCACTGTC GGCAGAGGCA

       6010       6020       6030       6040       6050       6060
     *    *     *    *     *    *     *    *     *    *     *    *
 TCTTGAACGA TAGCCTTTCC TTTATCGCAA TGATGGCATT TGTAGGTGCC ACCTTCCTTT

       6070       6080       6090       6100       6110       6120
     *    *     *    *     *    *     *    *     *    *     *    *
 TCTACTGTCC TTTTGATGAA GTGACAGATA GCTGGGCAAT GGAATCCGAG GAGGTTTCCC

       6130       6140       6150       6160       6170       6180
     *    *     *    *     *    *     *    *     *    *     *    *
 GATATTACCC TTTGTTGAAA AGTCTCAATA GCCCTTTGGT CTTCTGAGAC TGTATCTTTG

       6190       6200       6210       6220       6230       6240
     *    *     *    *     *    *     *    *     *    *     *    *
 ATATTCTTGG AGTAGACGAG AGTGTCGTGC TCCACCATGT TCCTCGACTC TAGAGGATCC

       6250       6260       6270       6280       6290       6300
     *    *     *    *     *    *     *    *     *    *     *    *
 CCGGGTACCG AGCTCGAATT CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC

       6310       6320       6330       6340       6350       6360
     *    *     *    *     *    *     *    *     *    *     *    *
 TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCT TTCGCCAGCT GGCGTAATAG

       6370       6380       6390       6400       6410       6420
     *    *     *    *     *    *     *    *     *    *     *    *
 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG GCGAATGGCG

       6430       6440       6450       6460       6470       6480
     *    *     *    *     *    *     *    *     *    *     *    *
 CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA TATGGTGCAC
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        6490      6500      6510      6520      6530      6540
   *    *    *    *    *    *    *    *    *    *    *    *
TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG CCCCGACACC CGCCAACACC

        6550      6560      6570      6580      6590      6600
   *    *    *    *    *    *    *    *    *    *    *    *
CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC

        6610      6620      6630      6640      6650
   *    *    *    *    *    *    *    *    *    *    *
CGTCTCCGGG AGCTGCATGT GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGA
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The sequence of pB2/35SAcK:

10 20 30 40 50
TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
60 70 80 90 100
GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
110 120 130 140 150
TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
160 170 180 190 200
CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCA AACAAACATA
210 220 230 240 250
CACAGCGACT TATGCTCAAA TTACAACGGT ATATATCCTG CCACATATGC
260 270 280 290 300
GGTGTGAAAT ACCGCACAGA TGCGTAAGGA GAAAATACCG CATCAGGCGC
310 320 330 340 350
CATTCGCCAT TCAGGCTGCG CAACTGTTGG GAAGGGCGAT CGGTGCGGGC
360 370 380 390 400
CTCTTCGCTA TTACGCCAGC TGGCGAAAGG GGGATGTGCT GCAAGGCGAT
410 420 430 440 450
TAAGTTGGGT AACGCCAGGG TTTTCCCAGT CACGACGTTG TAAAACGACG
460 470 480 490 500
GCCAGTGAAT TCCCATGGAG TCAAAGATTC AAATAGAGGA CCTAACAGAA
510 520 530 540 550
CTCGCCGTAA AGACTGGCGA ACAGTTCATA CAGAGTCTCT TACGACTCAA
560 570 580 590 600
TGACAAGAAG AAAATCTTCG TCAACATGGT GGAGCACGAC ACGCTTGTCT
610 620 630 640 650
ACTCCAAAAA TATCAAAGAT ACAGTCTCAG AAGACCAAAG GGCAATTGAG
660 670 680 690 700
ACTTTTCAAC AAAGGGTAAT ATCCGGAAAC CTCCTCGGAT TCCATTGCCC
710 720 730 740 750
AGCTATCTGT CACTTTATTG TGAAGATAGT GGAAAAGGAA GGTGGCTCCT
760 770 780 790 800
ACAAATGCCA TCATTGCGAT AAAGGAAAGG CCATCGTTGA AGATGCCTCT
810 820 830 840 850
GCCGACAGTG GTCCCAAAGA TGGACCCCCA CCCACGAGGA GCATCGTGGA
860 870 880 890 900
AAAAGAAGAC GTTCCAACCA CGTCTTCAAA GCAAGTGGAT TGATGTGATA
910 920 930 940 950
TCTCCACTGA CGTAAGGGAT GACGCACAAT CCCACTATCC TTCGCAAGAC
960 970 980 990 1000
CCTTCCTCTA TATAAGGAAG TTCATTTCAT TTGGAGAGGA CAGGGTACCC
1010 1020 1030 1040 1050
GGGGATCCAC CATGTCTCCG GAGAGGAGAC CAGTTGAGAT TAGGCCAGCT
1060 1070 1080 1090 1100
ACAGCAGCTG ATATGGCCGC GGTTTGTGAT ATCGTTAACC ATTACATTGA
1110 1120 1130 1140 1150
GACGTCTACA GTGAACTTTA GGACAGAGCC ACAAACACCA CAAGAGTGGA

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      1160       1170       1180       1190       1200
TTGATGATCT AGAGAGGTTG CAAGATAGAT ACCCTTGGTT GGTTGCTGAG
      1210       1220       1230       1240       1250
GTTGAGGGTG TTGTGGCTGG TATTGCTTAC GCTGGGCCCT GGAAGGCTAG
      1260       1270       1280       1290       1300
GAACGCTTAC GATTGGACAG TTGAGAGTAC TGTTTACGTG TCACATAGGC
      1310       1320       1330       1340       1350
ATCAAAGGTT GGGCCTAGGA TCCACATTGT ACACACATTT GCTTAAGTCT
      1360       1370       1380       1390       1400
ATGGAGGCGC AAGGTTTTAA GTCTGTGGTT GCTGTTATAG GCCTTCCAAA
      1410       1420       1430       1440       1450
CGATCCATCT GTTAGGTTGC ATGAGGCTTT GGGATACACA GCCCGGGGTA
      1460       1470       1480       1490       1500
CATTGCGCGC AGCTGGATAC AAGCATGGTG GATGGCATGA TGTTGGTTTT
      1510       1520       1530       1540       1550
TGGCAAAGGG ATTTTGAGTT GCCAGCTCCT CCAAGGCCAG TTAGGCCAGT
      1560       1570       1580       1590       1600
TACCCAGATC TGAGTCGACC TGCAGGCATG CCGCTGAAAT CACCAGTCTC
      1610       1620       1630       1640       1650
TCTCTACAAA TCTATCTCTC TCTATAATAA TGTGTGAGTA GTTCCCAGAT
      1660       1670       1680       1690       1700
AAGGGAATTA GGGTTCTTAT AGGGTTTCGC TCATGTGTTG AGCATATAAG
      1710       1720       1730       1740       1750
AAACCCTTAG TATGTATTTG TATTTGTAAA ATACTTCTAT CAATAAAATT
      1760       1770       1780       1790       1800
TCTAATTCCT AAAACCAAAA TCCAGTGGCG AGCTCGAATT CGAGCTCGGT
      1810       1820       1830       1840       1850
ACCCGGGGAT CCTCTAGAGT CGACCTGCAG GCATGCAAGC TTGGCGTAAT
      1860       1870       1880       1890       1900
CATGGTCATA GCTGTTTCCT GTGTGAAATT GTTATCCGCT CACAATTCCA
      1910       1920       1930       1940       1950
CACAACATAC GAGCCGGAAG CATAAAGTGT AAAGCCTGGG GTGCCTAATG
      1960       1970       1980       1990       2000
AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC GCTTTCCAGT
      2010       2020       2030       2040       2050
CGGGAAACCT GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG
      2060       2070       2080       2090       2100
AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC
      2110       2120       2130       2140       2150
GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG
      2160       2170       2180       2190       2200
CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG
      2210       2220       2230       2240       2250
TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT
      2260       2270       2280       2290       2300
GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC
      2310       2320       2330       2340       2350
GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
      2360       2370       2380       2390       2400
TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT
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      2410       2420       2430       2440       2450
TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
      2460       2470       2480       2490       2500
ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG
      2510       2520       2530       2540       2550
CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
      2560       2570       2580       2590       2600
CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC
      2610       2620       2630       2640       2650
TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
      2660       2670       2680       2690       2700
GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC
      2710       2720       2730       2740       2750
AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG
      2760       2770       2780       2790       2800
TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT
      2810       2820       2830       2840       2850
TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA
      2860       2870       2880       2890       2900
TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC
      2910       2920       2930       2940       2950
GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC
      2960       2970       2980       2990       3000
CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA
      3010       3020       3030       3040       3050
AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG
      3060       3070       3080       3090       3100
CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG
      3110       3120       3130       3140       3150
ATAACTACGA TACGGGAGGG CTTACCATCT GGCCCCAGTG CTGCAATGAT
      3160       3170       3180       3190       3200
ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC
      3210       3220       3230       3240       3250
CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC
      3260       3270       3280       3290       3300
ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT
      3310       3320       3330       3340       3350
TAATAGTTTG CGCAACGTTG TTGCCATTGC TACAGGCATC GTGGTGTCAC
      3360       3370       3380       3390       3400
GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG
      3410       3420       3430       3440       3450
CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG
      3460       3470       3480       3490       3500
TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA TCACTCATGG
      3510       3520       3530       3540       3550
TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC
      3560       3570       3580       3590       3600
TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG AATAGTGTAT
      3610       3620       3630       3640       3650
GCGGCGACCG AGTTGCTCTT GCCCGGCGTC AATACGGGAT AATACCGCGC
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      3660       3670       3680       3690       3700
CACATAGCAG AACTTTAAAA GTGCTCATCA TTGGAAAACG TTCTTCGGGG
      3710       3720       3730       3740       3750
CGAAAACTCT CAAGGATCTT ACCGCTGTTG AGATCCAGTT CGATGTAACC
      3760       3770       3780       3790       3800
CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTTT
      3810       3820       3830       3840       3850
CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG
      3860       3870       3880       3890       3900
GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC AATATTATTG
      3910       3920       3930       3940       3950
AAGCATTTAT CAGGGTTATT GTCTCATGAG CGGATACATA TTTGAATGTA
      3960       3970       3980       3990       4000
TTTAGAAAAA TAAACAAATA GGGGTTCCGC GCACATTTCC CCGAAAAGTG
      4010       4020       4030       4040       4050
CCACCTGACG TCTAAGAAAC CATTATTATC ATGACATTAA CCTATAAAAA
      4060       4070       4080       4090       4100
TAGGCGTATC ACGAGGCCCT TTCGTC.... .......... ..........
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Appendix 2. USDA Field Trial Termination Reports

List of Release Authorizations

Authorization Number	States and Sites
90-274-05	PR (1)
91-051-03	AR (1), IL (1), MD (1)
91-203-01	PR (1)
92-043-02	IA (1), IL (1), MS (1), NE (1)
92-043-03	AR (1), IL (1), MD (1)
92-308-01*	
93-090-01	MI (1)
93-047-02	AR (1), IL (1), MD (1)
93-047-03	IA (3), IL (3), NE (1)
93-120-31 (93-047-04)	IN (1), MO (1), ND (1), SC (1), VA (1)
93-120-35 (93-053-04)	AR (1), IA (1), IL (1), IN (2), MO (1), MS (1), VA (1)
93-127-02	IL (1)
93-270-03	PR (2)
94-080-03	AR (1), Fl (1), IA (7), IL (6), IN (3), KY (2), MD (1), MI (1), MN (2), MO (1), MS (1), NC (3), ND (3), NE (4), NJ (1), OH (4), PA (2), SD (1), TN (1), VA (1)
94-090-02	AR (1), IA (1), IL (1), IN (1), MD (1), WI (1)
94-131-01	IL (1)
95-034-02	MD (1), PR (1)
95-069-01	GA (4)
95-069-02	IA (17)
95-069-03	IL (10)
95-069-04	IN (8)
95-069-05	MN (4)
95-069-06	NC (4)
95-069-07	NE (4)
95-069-08	OH (7)
95-069-09	AL (1), AR (3), FL (1), LA (2), MS (3), SC (1), TN (4), VA (2)
95-069-10	KS (1), KY (2), MO (3), SD (2)
95-069-11	MD (1), NJ (1), PA (3)
95-069-12	MI (2), WI (4)
95-079-02	KS (1)
95-115-04	MO (1)
95-122-03	MD (1)
95-135-04	IA (2), MO (1)
95-142-02	AR (2), MS (1)

* Not planted

SUMMARY REPORT - PERMIT #90-274-05

As requested in the supplemental conditions included with the approval of permit #90-274-05, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans. The purpose of the trial was to increase the amount of seed for two transgenic stocks W62 and W98. The study was conducted from the fall of 1990 through spring 1991. Nineteen sublines from the two transgenic events (W62 and W98) were planted 12/19/90. The total area of the test plot was 3360 square ft. On 1/17/91, the soybean plants were sprayed with Ignite (1X = .45 lb ai/A or 2X = .9 lb ai/A) and segregation ratios noted. The seed which survived the spraying was harvested and transported to various stations in the US for future trials (permits were applied for and granted). Seed from non-transgenic soybean plants located within the border surrounding the transgenic plants were also harvested and shipped. These plants were were sprayed with 1X, 2X, and 4X levels if Ignite. Following is information regarding specific questions included in the supplemental conditions.

Any unexpected phenotypes?
The transgenic plants were carefully observed throughout the growing season and no abnormal phenotypes were observed for the transgenic plants. The transgenic plants were not unusually susceptible to any observable diseases or plant pests. The transgenic plants showed no phenotypic changes which might allow them to become a weed. No unusual change in overwintering habits was observed.

Any evidence of horizontal movement?
Seed from non-transgenic plants within the border were harvested and planted as part of our 1991 field trial (91-051-03). Listed in Table 1 are the number of surviving plants for each spray rate per location for non-transgenic border seed planted in this permit. Each cell in Table 1A had 1536 plants sprayed (4 reps) while each cell in Table 1B had 1152 plants sprayed (3 reps).

Table 1A
A3322 controls from IPBS border
Grown at QMBS
Number of surviving plants after spray w/ indicated Ignite dose

0X	1X	2X	4X
NA	4	0	0

A3322 controls from IPBS border
Grown at SIBS
Number of surviving plants after spray w/ indicated Ignite dose

0X	1X	2X	4X
NA	3	0	0

Table 1B
A5403 controls from IPBS border
Grown at QMBS
Number of surviving plants after spray w/ indicated Ignite dose

0X	1X	2X	4X
NA	2	0	0

A5403 controls from IPBS border
Grown at MABS
Number of surviving plants after spray w/ indicated Ignite dose

0X	1X	2X	4X
NA	0	0	0

The plants which survived the 1X treatment were severely stunted and produced no or very little seed. No plants survived the 2X or 4X rate. On the other hand, transgenic plants consistently show the ability to survive and thrive when sprayed with 2X and 4X concentrations of Ignite. Seed known to be susceptible (without any exposure to these transgenic lines) have on occasion survived 1X levels Ignite. We are confident, that these few survivors are in fact non-transgenic, and that there was no horizontal movement of pollen from the transgenic plants into the border. This was expected as soybeans are almost completely self-pollinated.

Stability and pattern of inheritance.

Plants from the R1 generation had previously been sprayed, leaving R2 seed from R1 plants that were either Res/Susc or Res/Res. One would expect R2 seed from these R1's to be either 75% tolerant, or 100% tolerant respectively. In Table 2 is listed the segregation ratios for each of the sublines. Several lines were classified as homozygous even though a small number of dead plants were observed. It is unclear, from this data, whether these dead plants resulted from occasional overexposure to the herbicide or variable expression of the Ignite tolerant gene. Progeny from other R1's showed segregation which appeared to be close to the expected 3:1 segregation; however, when subjected to Chi square analysis, was found to be significantly deviant from the expected ratio. At this point, it is unclear why this deviation has occurred. This situation will be monitored in future trials.

Table 2

	Subline	R:S Observed	R:S Observed	R:S Expected	Score
W62 R2 progeny from R1's classified as heterozygous	-4	329:90	690:188 P=.015	659:219	3
	-14	361:98			2
W62 R2 progeny from R1's classified as homozygous tolerant	-7	347:3	2586:49	2635:0	3
	-9	346:7			3
	-12	446:1			5
	-13	234:1			5
	-15	407:12			3
	-16	431:18			3
	-17	375:7			5
W98 R2 progeny from R1's classified as heterozygous	-2	283:69	1003:261 P=.001	948:316	1
	-9	230:61			2
	-17	246:70			4
	-18	244:61			5
W98 R2 progeny from R1's classified as homozygous tolerant.	-3	275:0	520:1	521:0	2
	-7	245:1			3

Expected R:S = 3:1 for single gene, dominant inheritance
P=probability that observed ratio is a random deviation of expected ratio.
Score=visual rating of surviving plants after spraying with 5=best and 1=worst.

Four sublines had both R2 and R3 seed planted and sprayed. The pattern of expression was very consistent across both generations. All four sublines appear to be homozygous for the resistant gene with a few plants consistently showing up as susceptible. This data suggests that expression has been consistent across generations, in the sense that a few plants, believed to be resistant, consistently are being killed. The most likely explanation would be overexposure of the occasional plants to the herbicide.

Stability of expression of tolerance over 2 generations

	R2 Seed		R3 Seed	
	R:S	Score	R:S	Score
W62-9	346:7	3	453:4	5
W62-12	446:1	5	231:2	4
W98-3	275:0	2	449:6	4
W98-7	245:1	3	466:2	3

1991 IGNITE TOLERANT SOYBEAN EXPERIMENTS

QUEENSTOWN, MARYLAND
MARION ARKANSAS
STONINGTON, ILLINOIS

USDA PERMIT # 91-051-03

Purpose of application

The purpose of the field trials was to (1) determine the level of tolerance of pCMC2114 transformed soybean plants to IGNITE (HOE 866 01H), (2) obtain agronomic data on these lines in a field environment, (3) backcross the transgenic lines to additional proprietary lines developed by Asgrow Seed Company, (4) test the performance of transgenic soybean plants under different culture conditions, involving different herbicides, and (5) increase and screen segregating populations to identify additional homozygous sublines for use in future studies.

Experimental Layout

These objectives were met through 4 separate experiments.

Experiment 1.

Experiment 1 was designed to examine the agronomic performance of W62 and W98 when sprayed with the herbicide Ignite. W62 resulted from a single insertion event from pCMC2114 into the Asgrow soybean variety A5403 (relative maturity group 5). This line was evaluated at Queenstown, Maryland and Marion, Arkansas. W98 resulted from a single insertion event from pCMC2114 into the Asgrow soybean variety A3322 (relative maturity group 3). This line was evaluated at Queenstown, Maryland and Stonington, Illinois.

Each plot consisted of four, 16 foot rows, with 128 seeds planted per row. There were 4 treatments involving 0X, 1X, 2X, and 4X (0, 2.5, 5.0, and 10.0 liters/ha respectively) levels of Ignite. These treatments were applied to several transgenic sublines and two non-transgenic controls. Sublines are sister lines, segregating from the same insertion event. For one replication of the experiment involving W98, there were 24 plots. For W62, there were 44 plots per replication (see table next page). For W98, three replications were employed in total and for W62, three replications were employed.

Experiment 1
Number of plots generated for 1 replication of W98

	0X IGNITE	1X IGNITE	2X IGNITE	4X IGNITE
W98-3 R2				
W98-7 R2				
W98-3 R3				
W98-7 R3				
Control				
Control				

Experiment 1
Number of plots generated for 1 replication of W62

	0X IGNITE	1X IGNITE	2X IGNITE	4X IGNITE
W62-7 R2				
W62-9 R2				
W62-9 R3				
W62-12 R2				
W62-12 R3				
W62-13 R2				
W62-15 R2				
W62-16 R2				
W62-17 R2				
Control				
Control				

No visual differences were observed during the growing season with the exception that the trial at Marion, Arkansas showed some yellowing during the week following spraying at the 2X and 4X rate and the trial at Queenstown, Maryland showed some yellowing for a week at the 4X rate. Non-transgenic plants died at all rates of application with the exception of a few isolated plants. There were no significant yield differences between lines or spray treatments in the transgenic materials. No differences were observed for maturity, height, and lodging. Seed from these trials were saved and either stored in a USDA inspected storage facility or shipped to Puerto Rico (Renewal of transport permit # 90-274-06).

Experiment 2 - Crossing block.

The purpose of experiment 2 was to backcross the Ignite tolerant trait into additional proprietary lines developed by Asgrow Seed Company to provide new material for future commercialization.

Pollen was collected from various transgenic plants (W62 and W98) and used for crosses on the nontransgenic lines. The F1 pods were harvested at maturity and the seeds were shipped to Puerto Rico (Renewal of transport permit #90-274-06).

Experiment 3 - Efficacy trial.

The purpose of experiment 3 was to compare the weed control of Ignite with various herbicide programs for soybeans. With the exception of Ignite, all other herbicides used were registered for use on soybeans.

Each plot in this experiment consisted of four, 16 foot rows, with 128 seeds planted per 16 foot row. For each replication, 10 plots of transgenic lines were planted and sprayed with various herbicides. The various herbicide regimes were evaluated for their effectiveness in weed control. Two of the 10 treatments involved a non-spray control and a commonly used herbicide system for soybeans. Eight of the 10 treatments involved treatments with Ignite. These treatments examined the effectiveness of Ignite, in various concentrations, and at various application dates. While we initially planned to plant W98 in Stonington, Illinois and Queenstown, Maryland and W62 in Queenstown, Maryland and Marion, Arkansas, we actually planted only W62 at Marion, AR and Queenstown, Md. Three replications of this experiment were planted at each location.

At the end of the season, the seed was harvested with a small plot harvestor and seed weight evaluated. Seed from this experiment was destroyed as described in the permit application.

Experiment 4 - Progeny row increase.

For the progeny row grow out, 1195 two row plots (5 ft long) were planted, sprayed and evaluated for segregation. Sublines of both W98 and W62 were both included in this grow out, with all sublines being derived from these two original insertion events. Rows were evaluated as either homozygous resistant or segregating. Segregating rows behaved as expected with approximately 3/4 of the row being tolerant. Seed from homozygous rows were harvested and stored in a USDA inspected storage facility. Seed from segregating rows were destroyed in the field as defined in the protocol.

SIGNIFICANT DATES

DATES	ILLINOIS	MARYLAND		ARKANSAS
	W98	W98	W62	W62
Planted	6/6/91	6/6/91	6/6/91	6/5/91
Sprayed	7/1/91	6/25/91	6/25/91	6/25/91
Harvested	10/18/91	10/9/91	11/5/91	10/10/91

GENERAL OBSERVATIONS

All plots grew normally during the course of the experiment. Except for some minor somaclonal variation in some lines, no obvious differences in growth or yield could be detected between the unsprayed transgenic and nontransgenic plants. No plant damage was observed that could be attributed to birds or rodents, and the plants and remaining seed were either harvested and stored or destroyed according to the protocol. Nothing unusual was observed during the course of the experiment.

RESPONSES TO SPECIFIC ISSUES.

1. Horizontal Movement.
   No weed species or other crops that could outcross with soybeans were present in the experimental area, so that transfer of the gene to other species through outcrossing was not possible. As required in the protocol, the plants were spaced at least 20 ft. away from other soybean plants, so that there was little chance of outcrossing or seed mixture with other soybean plants.

2. Changes in survival characteristics.
   There was no evidence of changes in the survival characteristics of the transgenic soybean plants. The plots were monitored on a regular basis over the winter and the next spring, and no new growth could be observed.

3. Stability and inheritance of the new genes.
   The transgenic lines included in the experiments were either homozygous resistant, or segregating. Homozygous susceptible rows were eliminated by spraying plants from the previous generation with Ignite. Segregation patterns were indicative of those expected for a single, dominant gene. It was not unusual to see a plant or two die in a row characterized as homozygous resistant. When progeny from these rows were replanted and sprayed, they maintained the tolerant phenotype.

4. Published data.
   There are no publications resulting from these experiments.

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT: #91-203-01

Renewal of: #90-274-05

Permittee: Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road Kalamazoo, MI 49002-1782; (616) 384-5531

Date of Release: November 14 1991

Site Contact: Ms. Yolanda Otero-Ortiz, Asgrow Seed Company

Site of Release: Isabela, PR

Purpose: To advance generations and increase glufosinate tolerant soybeans.

Results: Advancement of one generation was made and seed was increased.

As requested in the supplemental conditions included with the approval of permit #91-203-01, I am submitting a summary of the field trial involving our Ignite tolerant soybeans at supervised by Ms. Yolanda Otero-Ortiz, Asgrow Seed Co, at Isabela, PR,. The purpose of the trial was to grow bulks of transgenic soybeans for further evaluation. Only one generation requested in the original permit was grown. The seed used in these studies were transformed versions of grow soybean varieties A3322 (known as W98) and A5403 (known as W62). Twenty-eight plots in total of the two insgenic soybeans were grown.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests. There were White Flies, Weevils and Chinch Bugs present at that location.

Pesticide Tolerance: Ignite was not sprayed in this trial. Conventional soybean herbicides were used with no differences in sensitivity seen between transformed and non-transformed soybeans.

Date of Release Termination: March 5, 1992.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the plot was monitored and left unplanted for more than 5 months . The following year, the field was rotated out of soybeans. The field was monitored for a whole year and any volunteer soybean plants were destroyed.

Means of Plant Disposition: The harvested seed was stored on site in a cold room until it was shipped to the continental US under permit. The non-harvested transgenic residue was disposed of by incorporating it into the soil.

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate-Ammonium Tolerance

PERMIT: #92-043-02

Permittee:
Dr. Ted Diedrick,
Asgrow Seed Company,
2605 E. Kilgore Road
6825-248-013
Kalamazoo, MI 49002-1744
(616) 384-5531

Report Author:
Dr. John McGregor
AgrEvo USA Company
PO Box 164
Wonder Lake, IL 60097

Date of Release: June 1992

Dates of Termination: October through November, 1992

Sites of Release:
(States/Number per state)
Illinois/1, Iowa/1, Mississippi/1
Nebraska/1.

Purpose of Release:
evaluate weed control with glufosinate-ammonium herbicide when applied to soybean plants containing the BAR gene which confers tolerance to glufosinate-ammonium herbicide. The soybean varieties in this release were varieties from Asgrow Seed Company.

Results:
Glufosinate-ammonium herbicide provided control of economically important weeds in soybeans with no injury to the transgenic soybean plants.

Observations:
The frequency of observations differed with each location. Each location was visited an average of five times during the duration of the release. The area planted to the transgenic soybeans ranged from .11 to .15 acres per site. The transgenic soybean planting rate was approximately 140,000 seeds per acre.

Herbicide tolerance: The transgenic soybean plants exhibited tolerance to glufosinate-ammonium herbicide. The transgenic soybean plants were also tolerant to other commercially used soybean herbicides that were used in the trials as standards. The nontransgenic soybeans were severely injured by treatment with glufosinate-ammonium.

Disease and Insect Susceptibility: Diseases in soybean production are sporadic and are often associated with environmental conditions. Observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybeans.

Weather Related Conditions: Across all the regions where the trials were conducted, the weather patterns during the growing season were normal. The transgenic and nontransgenic soybeans responded identically to weather conditions.

Physical Characteristics: The soybean plants were observed from emergence through maturity. No differences were observed between transgenic and nontransgenic soybeans in emergence, seedling vigor and stand establishment. Prior to glufosinate-ammonium application, no morphological differences were observed between the transgenic and non-transgenic plants. After glufosinate-ammonium application, the transgenic plants continued to grow normally. The nontransgenic soybean was severely injured by glufosinate-ammonium.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and nontransgenic ınts. Weediness characteristics such as excessive vegetative growth or seed shattering were not present.

Means of plant disposition:
The destruction of the plants differed by site and consisted of mechanical mowing, burning, disking, and/or plowing.

Time/Methods of monitoring for volunteers:
Sites were visited one or more times the following spring when soil temperatures reached a level at which soybean emergence may be expected. The sites were visually inspected for volunteer soybean plants.

Number of volunteers observed/action taken:
The number of volunteers ranged from none, to numbers which would be expected in commercial soybean production. Soybean seeds typically degrade in or on the soil surface under normal weather conditions. It is important to note that the population makeup of the volunteers may have contained an equal number of nontransgenic and transgenic plants. This can be attributed to the fact that the nontransgenic rows were allowed to reach maturity and the seed were incorporated into the soil. All volunteer soybean plants were destroyed by mechanical means, removed by hand, or destroyed with herbicides other than glufosinate-ammonium.

⁻'JMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT:	#92-043-03
Permittee:	Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road Kalamazoo, MI 49002-1782; (616) 384-5531
Date of Release:	May 1992
Site Contact:	Dr. Craig Moots, Asgrow Seed Company
Site of Release:	Stonington, IL
Purpose:	To evaluate agronomics and efficacy of glufosinate tolerant soybeans.
Results:	Qualitative and quantitative comparisons between glufosinate tolerant and non-transgenic soybeans.

As requested in the supplemental conditions included with the approval of permit #92-043-03, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Dr. Craig Moots, Asgrow Seed Co, at Stonington, IL,. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A3322 (known as W98).

. ..enotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant disease and insect pests present at that location.

Pesticide Tolerance: Ignite and Lasso herbicide was the only herbicides applied. There were no differences in sensitivity to Lasso. As expected the transgenic soybeans were much more tolerant of Ignite than the non-transgenic soybeans. In this trial 350 g a.i. glufosinate was enough to kill the non-transgenic soybeans.

Date of Release Termination: October 30, 1992.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the plot was monitored and left unplanted for more than 5 months . The following year, the field was rotated out of soy beans. The field was monitored for a whole year and any volunteer soybean plants were destroyed.

Inspection: The field was inspected by USDA inspector Bill Winnie on June 10, 1992.

Means of Plant Disposition: The harvested seed was either stored on site or disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil by disking.

#92-043-03

Site Contact: Dr. Chris Tinius, Asgrow Seed Company

Date of Release: May 1992

Site of Release: Marion, AR

Purpose: To evaluate agronomics and efficacy of glufosinate tolerant soybeans.

Results: Qualitative and quantitative comparisons between glufosinate tolerant and non-transgenic soybeans.

As requested in the supplemental conditions included with the approval of permit #92-043-03, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Dr. Chris Tinius, Asgrow Seed Co, at Marion, AR,. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A5403 (known as W62).

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant levels of disease present at that location. There was Soybean Cyst Nematodes present but the transgenic soybeans did not react any differently than non-transgenic soybeans.

Pesticide Tolerance: Ignite was the only herbicide applied. As expected the transgenic soybeans were much more tolerant of Ignite than the non-transgenic soybeans. In this trial less than 500 g a.i. glufosinate was enough to kill the non-transgenic soybeans.

Date of Release Termination: October, 1992.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months . The following year, the field was rotated out of soybeans.

Inspection: The field was inspected by a BPEP inspector.

Means of Plant Disposition: The harvested seed was either stored on site, shipped to another destination under permit or disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil.

Site Contact: Billy Rhodes, Asgrow Seed Company

Date of Release: June 1992

Site of Release: Galena, MD

Purpose: To evaluate agronomics and efficacy of glufosinate tolerant soybeans.

Results: Qualitative and quantitative comparisons between glufosinate tolerant and non-transgenic soybeans.

#92-043-03

s requested in the supplemental conditions included with the approval of permit #92-043-03, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Billy Rhodes, Asgrow Seed Co, at Galena, MD. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A5403 (known as W62) and A3322 (known as W98).

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant disease and insect pests present at that location.

Pesticide Tolerance: Ignite, Dual and Scepter herbicide were the only herbicides applied. There were no differences in sensitivity to Dual or Scepter. As expected the transgenic soybeans were much more tolerant of Ignite than the non-transgenic soybeans. In this trial 150 g a.i. glufosinate was enough to kill the non-transgenic soybeans.

Date of Release Termination: August 24, 1992.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. The plosts were not harvested for seed but mowed down and disked. The plot was monitored and left unplanted for more than 5 months . The following year, the field was rotated out of soy beans. The field was monitored for a whole year and any volunteer soybean plants were destroyed.

Inspection: The field was inspected by both BPEP and a Regional Biotechnologist.

Means of Plant Disposition: There was no harvested seed at this site. The non-harvested transgenic plant material was disposed of by incorporating it into the soil by disking on August 24, 1992.

~~S~~UMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT: #92-090-01

Permittee: Dr. Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road
Kalamazoo, MI 49002-1782; (616) 384-5531
FAX (616) 384-5646

Date of Release: July 27,1992

Site Contact: Dr. Ted Diedrick, Asgrow Seed Company

Site of Release: Kalamazoo, MI

Purpose: To demonstrate glufosinate tolerant soybeans.

Results: Qualitative comparisons between glufosinate tolerant and non-transgenic soybeans demonstrated to sales personnel and growers.

As requested in the supplemental conditions included with the approval of permit #92-090-01, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at Kalamazoo, MI, supervised by Dr. Ted Diedrick, Asgrow Seed Co. The purpose of the trial was to demonstrate the performance of transgenic soybean plants containing the phosphoinothricin acetyltransferase (PAT) gene with the herbicide IGNITE. The seed used in these studies were transformed versions of Asgrow soybean varieties. For the purposes of demonstration, the ts were planted almost 2 months after ideal soybean planting and thus no viable seed was set.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed. Due to the late planting, no seed was set on either transgenic or non-transgenic soybeans.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant diseases present at that location. There was equal feeding by Japanese Beetles on both transgenic and non-transgenic plants.

Herbicide Tolerance: Date of Ignite spraying was August 24, 1992. There was no other pesticides applied. As expected the transgenic soybeans were much more tolerant of Ignite.

Date of Release Termination: September 14, 1992.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. Additionally, due to the late planting, there were no other soybeans flowering at this date. Due to the lack of seed set, there were no volunteer soybeans produced. The field was continuously monitored each month and left unplanted for more than 5 months. The following year, the field was rotated out of soybeans.

Inspection: The field inspected by Regional Biotechnologist in October, 1992 and in the spring of 1993.

Means of Plant Disposition: There was no harvested seed as no seed was set. No transgenic soybean material was removed from the demonstration area. All soybean material was incorporated directly into the test site by rototilling September 14, 1992.

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT:	#92-308-01
Permittee:	Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road Kalamazoo, MI 49002-1782; (616) 384-5531
Purpose:	To make crosses between Asgrow elite germplasm and glufosinate transgenic soybeans; to advance generations and increase material
Results:	No field trials were undertaken under this permit
Site of Intended Release:	Isabela, PR
Date of Release:	Not released
Site Contact:	Yolanda Otero-Ortiz, Asgrow Seed Company

As requested in the conditions included with the approval of permit #92-308-01, I am submitting a final report. Because we did not need to increase our stocks of Ignite tolerant soybeans, the field trials covered by this permit were never undertaken.

~UMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT: #93-047-02

Permittee: Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road
Kalamazoo, MI 49002-1782; (616) 384-5531
FAX (616) 384-5646

Purpose: To evaluate agronomics and efficacy of glufosinate tolerant soybeans.

Results: Qualitative and quantitative comparisons between glufosinate tolerant and non-transgenic soybeans.

Site Contact: Dr. Craig Moots, Asgrow Seed Company

Date of Release: May 1993

Site of Release: Stonington, IL

As requested in the supplemental conditions included with the approval of permit #93-047-02, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Dr. Craig Moots, Asgrow Seed Co, at Stonington, IL,. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. ıe seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A3322 (known as .√98).

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant disease and insect pests present at that location.

Pesticide Tolerance: Ignite and Lasso herbicide was the only herbicides applied. There were no differences in sensitivity to Lasso. As expected the transgenic soybeans were much more tolerant of Ignite than the non-transgenic soybeans. In this trial 350 g a.i. glufosinate was enough to kill the non-transgenic soybeans.

Date of Release Termination: November 19, 1993.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the plot was monitored and left unplanted for more than 5 months . The following year, the field was rotated out of soy beans. The field was monitored for a whole year and any volunteer soybean plants were destroyed.

Inspection: The field was inspected by a Regional Biotechnologist.

Means of Plant Disposition: The harvested seed was either stored on site or disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil by sking.

Site Contact: Dr. Chris Tinius, Asgrow Seed Company

Site of Release: Marion, AR

Date of Release: June, 1993

As requested in the supplemental conditions included with the approval of permit #93-047-02, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Dr. Chris Tinius, Asgrow Seed Co, at Marion, AR. The purpose of the trial was to test the stability and agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A5403 (known as W62).

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed. Segregation data on F2 populations of 3 sublines was collected (Table 1). The expected 3:1 ratio was not realized over all three sublines, although within individual populations there were some that fit the single dominant gene model. We are continuing to evaluate these sublines to determine if there might be multiple gene insertions or other factors affecting the segregation ratios.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant disease present at that location. There was Soybean Cyst Nematodes present but there were no apparent differences between the transgenic and non-transgenic plants.

Pesticide Tolerance: Ignite was the only herbicides applied. . As expected the transgenic soybeans were much more tolerant of Ignite than the non-transgenic soybeans. In this trial less than 500 g a.i. glufosinate was enough to kill the non-transgenic soybeans.

Date of Release Termination: October, 1993.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the plot was monitored two months post-harvest and left unplanted for more than 5 months.

Inspection: The field was inspected by a BPEP inspector.

Means of Plant Disposition: The harvested seed was either stored on site, shipped under permit to another desitnation or disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil by disking.

Site Contact: Bill Rhodes, Asgrow Seed Company

Date of Release: May 1993

Site of Release: Galena, MD

As requested in the supplemental conditions included with the approval of permit #93-047-02, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Bill Rhodes, Asgrow Seed Co, at Galena MD. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used these studies were homozygous transformed versions of Asgrow soybean varieties A5403 (known as W62).

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared

-n non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a -eed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant disease and insect pests present at that location.

Pesticide Tolerance: Ignite and Blazer herbicide was the only herbicides applied. There were no differences in sensitivity to Blazer. As expected the transgenic soybeans were much more tolerant of Ignite than the non-transgenic soybeans. In this trial 150 g a.i. glufosinate was enough to kill the non-transgenic soybeans.

Date of Release Termination: November 16, 1993.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the plot was monitored and left unplanted for more than 5 months . The following year, the field was rotated out of soy beans. The field was monitored for a whole year and any volunteer soybean plants were destroyed.

Inspection: The field was inspected by a BPEP and a Regional Biotechnologist.

Means of Plant Disposition: The harvested seed was either shipped to another site under permit or disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil by disking.

-able 1. Marion, AR; 1994;Reaction of F_2 populations to Ignite (500 g/ha) and goodness-of-fit test to 3 alive: 1 dead -pected ratio (Single dominant gene model)

Cross	Dead	Alive	Total	X^2	P
Asgrow Elite * W62-07R2	416	1944	2360	68.4	<<0.005
Asgrow Elite * W62-15R2	253	1147	1400	35.9	<<0.005
Asgrow Elite * W62-17R2	96	425	521	12.0	<0.005
All populations	765	3516	4281	>115	<<<0.005

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate-Ammonium Tolerance

PERMIT: #93-047-03

Permittee: Dr. Ted Diedrick,
Asgrow Seed Company,
2605 E. Kilgore Road
6825-248-013
Kalamazoo, MI 49002-1744
(616) 384-5531

Report Author: Dr. John McGregor
AgrEvo USA Company
PO Box 164
Wonder Lake, IL 60097

Date of Release: May through June 1992

Dates of Termination: July through November, 1992

Sites of Release: (States/Number per state)
Illinois/3, Iowa/3, Nebraska/1.

Purpose of Release:
To evaluate weed control with glufosinate-ammonium herbicide when applied to soybean plants containing the BAR gene which confers tolerance to glufosinate-ammonium herbicide. The soybean varieties in this release were varieties from Asgrow Seed Company.

Results:
Glufosinate-ammonium herbicide provided control of economically important weeds in soybeans with no injury to the transgenic soybean plants.

Observations:
The frequency of observations differed with each location. Each location was visited an average of five times during the duration of the release. The area planted to the transgenic soybeans ranged from .07 to .70 acres per site. The transgenic soybean planting rate was approximately 148,000 seeds per acre.

Herbicide tolerance: The transgenic soybean plants exhibited tolerance to glufosinate-ammonium herbicide. The transgenic soybean plants were also tolerant to other commercially used soybean herbicides that were used in the trials as standards. The nontransgenic soybeans were severely injured by treatment with glufosinate-ammonium.

Insect Susceptibility: Damaging levels of insect pests were not observed at any locations on either transgenic or nontransgenic soybeans.

Disease and Insect Susceptibility: Diseases in soybean production are sporadic and are often associated with environmental conditions. Observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybeans.

Weather Related Conditions: The majority of the sites were located in the midwestern United States which was subjected to excessive amounts of

rainfall with associated flooding. At some locations, the soybean plants were subjected to temporary flooding and water logged soils. Trials at one location were terminated due to persistent flooding. There was no differences in the response of the transgenic and non-transgenic plants to the excessive rainfall.

Physical Characteristics: The soybean plants were observed from emergence through maturity. No differences were observed between transgenic and nontransgenic soybeans in emergence, seedling vigor and stand establishment. Prior to glufosinate-ammonium application, no morphological differences were observed between the transgenic and non-transgenic plants. After glufosinate-ammonium application, the transgenic plants continued to grow normally. The nontransgenic soybean was severely injured by glufosinate-ammonium.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and nontransgenic plants. Weediness characteristics such as excessive vegetative growth or seed shattering were not present.

Means of plant disposition:
The destruction of the plants differed by site and consisted of mechanical mowing, burning, disking, and/or plowing.

Time/Methods of monitoring for volunteers:
Sites were visited one or more times the following spring when soil temperatures reached a level at which soybean emergence may be expected. The sites were visually inspected for volunteer soybean plants.

Number of volunteers observed/action taken:
The number of volunteers ranged from none, to numbers which would be expected in commercial soybean production. Soybean seeds typically degrade in or on the soil surface under normal weather conditions. It is important to note that the population makeup of the volunteers may have contained an equal number of nontransgenic and transgenic plants. This can be attributed to the fact that the nontransgenic rows were allowed to reach maturity and the seed were incorporated into the soil. All volunteer soybean plants were destroyed by mechanical means, removed by hand, or destroyed with herbicides other than glufosinate-ammonium.

UMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate-Ammonium Tolerance

PERMIT: #93-047-04 (notification #93-120-31)

Permittee: Dr. Ted Diedrick,
Asgrow Seed Company,
2605 E. Kilgore Road
6825-248-013
Kalamazoo, MI 49002-1744
(616) 384-5531

Report Author: Dr. John McGregor
AgrEvo USA Company
PO Box 164
Wonder Lake, IL 60097

Date of Release: May through June 1992

Dates of Termination: July through November, 1992

Sites of Release: (States/Number per state)
Indiana/1, North Dakota/1, South Carolina/1, Missouri/1, Virginia/1.

Purpose of Release:
To evaluate weed control with glufosinate-ammonium herbicide when applied to soybean plants containing the BAR gene which confers tolerance to glufosinate-ammonium herbicide. The soybean varieties in this release were varieties from Asgrow Seed Company.

Results:
Glufosinate-ammonium herbicide provided control of economically important weeds in soybeans with no injury to the transgenic soybean plants.

Observations:
The frequency of observations differed with each location. Each location was visited an average of five times during the duration of the release. The area planted to the transgenic soybeans ranged from .07 to .70 acres per site. The transgenic soybean planting rate was approximately 148,000 seeds per acre.

Herbicide tolerance: The transgenic soybean plants exhibited tolerance to glufosinate-ammonium herbicide. The transgenic soybean plants were also tolerant to other commercially used soybean herbicides that were used in the trials as standards. The nontransgenic soybeans were severely injured by treatment with glufosinate-ammonium.

Insect Susceptibility: Damaging levels of insect pests were not observed at any locations on either transgenic or nontransgenic soybeans.

Disease and Insect Susceptibility: Diseases in soybean production are sporadic and are often associated with environmental conditions. Observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybeans.

Weather Related Conditions: The majority of the sites were located in the

ıidwestern United States which was subjected to excessive amounts of rainfall with associated flooding. At some locations, the soybean plants were subjected to temporary flooding and water logged soils. Trials at one location were terminated due to persistent flooding. There was no differences in the response of the transgenic and non-transgenic plants to the excessive rainfall.

Physical Characteristics: The soybean plants were observed from emergence through maturity. No differences were observed between transgenic and nontransgenic soybeans in emergence, seedling vigor and stand establishment. Prior to glufosinate-ammonium application, no morphological differences were observed between the transgenic and non-transgenic plants. After glufosinate-ammonium application, the transgenic plants continued to grow normally. The nontransgenic soybean was severely injured by glufosinate-ammonium.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and nontransgenic plants. Weediness characteristics such as excessive vegetative growth or seed shattering were not present.

Means of plant disposition:
The destruction of the plants differed by site and consisted of mechanical mowing, burning, disking, and/or plowing.

Time/Methods of monitoring for volunteers:
Sites were visited one or more times the following spring when soil temperatures reached a level at which soybean emergence may be expected. The sites were visually inspected for volunteer soybean plants.

Number of volunteers observed/action taken:
The number of volunteers ranged from none, to numbers which would be expected in commercial soybean production. Soybean seeds typically degrade in or on the soil surface under normal weather conditions. It is important to note that the population makeup of the volunteers may have contained an equal number of nontransgenic and transgenic plants. This can be attributed to the fact that the nontransgenic rows were allowed to reach maturity and the seed were incorporated into the soil. All volunteer soybean plants were destroyed by mechanical means, removed by hand, or destroyed with herbicides other than glufosinate-ammonium.

UMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate-Ammonium Tolerance

PERMIT: #93-053-04 (notification #93-120-35)

Permittee: Dr. Ted Diedrick,
Asgrow Seed Company,
2605 E. Kilgore Road
6825-248-013
Kalamazoo, MI 49002-1744
(616) 384-5531

Report Author: Dr. John McGregor
AgrEvo USA Company
PO Box 164
Wonder Lake, IL 60097

Date of Release: May through June 1992

Dates of Termination: July through November, 1992

Sites of Release: (States/Number per state)
Illinois/1, Iowa/1, Arkansas/1, Indiana/2, Missouri/1, Mississippi/1, Virginia/1.

Purpose of Release:
To evaluate weed control with glufosinate-ammonium herbicide when applied to soybean plants containing the BAR gene which confers tolerance to glufosinate-ammonium herbicide. The soybean varieties in this release were varieties from Asgrow Seed Company.

Results:
Glufosinate-ammonium herbicide provided control of economically important weeds in soybeans with no injury to the transgenic soybean plants.

Observations:
The frequency of observations differed with each location. Each location was visited an average of five times during the duration of the release. The area planted to the transgenic soybeans ranged from .07 to .70 acres per site. The transgenic soybean planting rate was approximately 148,000 seeds per acre.

Herbicide tolerance: The transgenic soybean plants exhibited tolerance to glufosinate-ammonium herbicide. The transgenic soybean plants were also tolerant to other commercially used soybean herbicides that were used in the trials as standards. The nontransgenic soybeans were severely injured by treatment with glufosinate-ammonium.

Insect Susceptibility: Damaging levels of insect pests were not observed at any locations on either transgenic or nontransgenic soybeans.

Disease and Insect Susceptibility: Diseases in soybean production are sporadic and are often associated with environmental conditions. Observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybeans.

Weather Related Conditions: The majority of the sites were located in the

idwestern United States which was subjected to excessive amounts of rainfall with associated flooding. At some locations, the soybean plants were subjected to temporary flooding and water logged soils. Trials at one location were terminated due to persistent flooding. There was no differences in the response of the transgenic and non-transgenic plants to the excessive rainfall.

Physical Characteristics: The soybean plants were observed from emergence through maturity. No differences were observed between transgenic and nontransgenic soybeans in emergence, seedling vigor and stand establishment. Prior to glufosinate-ammonium application, no morphological differences were observed between the transgenic and non-transgenic plants. After glufosinate-ammonium application, the transgenic plants continued to grow normally. The nontransgenic soybean was severely injured by glufosinate-ammonium.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and nontransgenic plants. Weediness characteristics such as excessive vegetative growth or seed shattering were not present.

Means of plant disposition:
The destruction of the plants differed by site and consisted of mechanical mowing, burning, disking, and/or plowing.

Time/Methods of monitoring for volunteers:
Sites were visited one or more times the following spring when soil temperatures reached a level at which soybean emergence may be expected. The sites were visually inspected for volunteer soybean plants.

Number of volunteers observed/action taken:
The number of volunteers ranged from none, to numbers which would be expected in commercial soybean production. Soybean seeds typically degrade in or on the soil surface under normal weather conditions. It is important to note that the population makeup of the volunteers may have contained an equal number of nontransgenic and transgenic plants. This can be attributed to the fact that the nontransgenic rows were allowed to reach maturity and the seed were incorporated into the soil. All volunteer soybean plants were destroyed by mechanical means, removed by hand, or destroyed with herbicides other than glufosinate-ammonium.

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT: #93-127-02

Permittee: Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road
Kalamazoo, MI 49002-1782; (616) 384-5531

Purpose: To evaluate efficacy and agronomics of glufosinate tolerant soybeans.

Results: Qualitative comparisons between glufosinate tolerant and non-transgenic soybeans.

Site of Release: Douglas County, IL

Date of Release: May 1993

Site Contact: Kevin Coey, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #93-127-02, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at Douglas County, IL, supervised by Kevin Coey, Asgrow Seed Co. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A3322 (known as W98).

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant disease and insect pests present at that location.

Herbicide Tolerance: Date of Ignite spraying was July 2, 1993. There was no other pesticides applied.

Date of Release Termination: September 14, 1993.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the plot was cultivated 2 months post harvest and left unplanted for more than 5 months . The following year, the field was rotated out of soy beans. The field was monitored for a whole year and any volunteer soybean plants were destroyed.

Inspection: The field inspected by a Regional Biotechnologist.

Means of Plant Disposition: The harvested seed disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil.

Site of Release: McLean County, IL

Date of Release: June, 1993

Site Contact: Brian Freed, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #93-127-02, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at McLean County, IL, supervised by Brian Freed, Asgrow Seed Co. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A3322 (known as W98).

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant levels of disease and insect pests present at that location.

Herbicide Tolerance: Date of Ignite spraying was August 2, 1993. There was no other pesticides applied.

Date of Release Termination: October, 1993.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants left unplanted for more than 5 months . The following year, the field was rotated out of soy beans. The field was monitored for a whole year and any volunteer soybean plants were destroyed.

Inspection: The field inspected by a Regional Biotechnologist and a BPEP inspector.

Means of Plant Disposition: The seed was not harvested. The transgenic soybeans were disposed of by incorporating them into the soil.

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT:	#93-270-03N
Permittee:	Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road Kalamazoo, MI 49002-1782; (616) 384-5531
Purpose:	To advance generations and increase material of glufosinate tolerant transgenic soybeans;
Results:	Generations were advanced and material increased.
Site of Intended Release:	Isabela, PR and Juana Diaz, PR.
Date of Initial Release:	November, 1993
Date of Release Termination:	April, 1994
Site Contact:	Yolanda Otero-Ortiz, Asgrow Seed Company

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant levels of disease present at that location. There were White Flies, Weevils and Chinch Bugs present but the transgenic soybeans did not appear more susceptible than non-transgenic soybeans.

Pesticide Tolerance: Ordinary soybean herbicides were applied. There was no apparent difference between Ignite tolerant and Ignite sensitive soybeans in their sensitivity to the other herbicides.

Inspection: Fields were inspected by Department of Agriculture inspector in August, 1994.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months and cultivated post-harvest. The field will be rotated out of soybeans in 1995. The site is continuously monitored for volunteers.

Means of Plant Disposition: The harvested seed was either stored on site for replanting, shipped to the US under permit or disposed of by returning it to the site and incorporating it into the soil. Transgenic seed was also shipped under permit to the continental USA. The non-harvested transgenic residue was disposed of by incorporating it into the soil.

SUMMARY REPORT TO THE FIELD RELEASE OF TRANSGENIC SOYBEAN EXPRESSING RESISTANCE TO THE HERBICIDE GLUFOSINATE

DATE OF REPORT: November 8, 1995

NOTIFICATION NUMBER: 94-080-03N

APPLICANT: Dr. Ed Pieters
AgrEvo USA Company
2711 Centerville Road
Wilmington, DE 19808

DATES OF RELEASE: April through August 1994

DATES OF TERMINATION: July through November 1994

SITES OF RELEASE (States/Number per State): Arkansas/1, Florida/1, Illinois/6, Indiana/3, Iowa/7, Kentucky/2, Maryland/1, Michigan/1, Minnesota/2, Mississippi/1, Missouri/1, New Jersey/1, North Carolina/3, North Dakota/3, Nebraska/4, Ohio/4, Pennsylvania/2, South Dakota/1, Tennessee/1, Virginia/1.

PURPOSE OF RELEASE

To evaluate weed control with glufosinate herbicide when applied to soybean plants containing the *bar* gene which confers resistance to glufosinate herbicide. The transgenic material was W98 derived from Asgrow 3222 variety maturity group 3 for midwest soybean production and W98 derived from Asgrow 5403 variety maturity group 5 for southern soybean production.

RESULTS

Glufosinate herbicide provided control of economically important weeds in soybean with no injury to the transgenic soybean plants.

OBSERVATIONS

The frequency of observations differed with each location. Each location was visited an average of three times (range of one to eight) during the duration of the release. The area planted to transgenic soybean ranged from 0.3 to 2.0 acres per site. The transgenic soybean population was an average of 150,000 plants per acre.

Herbicide Tolerance: The transgenic soybean plants exhibited resistance to glufosinate herbicide. The transgenic soybean plants were also tolerant to other commercially used soybean herbicides that were used in the trials as standards. The nontransgenic soybean was severely injured by treatment with glufosinate.

Herbicide Susceptibility: Trials were conducted to demonstrate that there was no cross tolerance in glufosinate resistant plants to other herbicides. The transgenic soybean plants were sensitive to glyphosate and dicamba (herbicides not registered for use on soybean that belong to a different chemical class than glufosinate). Death of the plants resulted when these herbicides were applied to transgenic soybean.

Insect Susceptibility: The primary insect pests of soybean are green cloverworm, soybean loopers, stink bugs, and leafhoppers. Pest infestations of green cloverworm, soybean loopers, stink bugs, leafhoppers, bean leaf beetles, grasshoppers, and whitefly were observed at release sites. There were no differences between transgenic and nontransgenic soybean. Lady beetles and pirate bugs were common beneficial insects observed at test sites, but no differences in population levels were observed on transgenic or nontransgenic soybean.

Disease Susceptibility: Asgrow 3222 W98 Group 3 soybean are moderately resistant to brown stem rot, resistant to phytophthora, and susceptible to soybean cyst nematode. Asgrow 5403 W62 Group 5 soybean are susceptible to sudden death syndrome and phytophthora, moderately susceptible to frog eye, and resistant to soybean cyst nematode and stem canker. No deterioration of the resistant traits have been observed over the past three years. Casual observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybean.

Weather Related Conditions: The weather conditions were ideal for soybean production. the exceptions were the Ohio and Maryland sites where below average rainfall occurred early in the year.

Physical Characteristics: The soybean plants were observed from emergence through maturity. No differences were observed between transgenic and nontransgenic soybean in emergence, seedling vigor, and stand establishment. Prior to glufosinate application no morphological differences were observed between the transgenic and non-transgenic plants. After glufosinate application, the transgenic plants continued to grow normally. The nontransgenic soybean was severely injured by glufosinate.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and nontransgenic pants.

MEANS OF PLANT DISPOSITION
The destruction of the plants differed by site and consisted of mechanical mowing, disking, land fill, and/or plowing.

NUMBER OF VOLUNTEERS OBSERVED/ACTION TAKEN
Sites were visited one or more times in the spring of 1995 when soil temperatures reached a level when soybean emergence occurred. No volunteers were noted. Regardless, in many of the sites, cultivation and/or application of herbicides that would normally destroy soybeans, were conducted to eliminate any volunteers that might possibly emerge.

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT: #94-090-02N

Permittee: Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road
Kalamazoo, MI 49002-1782; (616) 384-5531
FAX (616) 384-5646

Purpose: To make crosses between Asgrow elite germplasm and glufosinate tolerant transgenic soybeans; to evaluate efficacy of Glufosinate. To advance breeding material; Demonstration plots.

Results: Qualitative and quantitative comparisons between glufosinate tolerant and non-transgenic soybeans. Soybean crosses were made. Segregating material was evaluated and generations were advanced. Segregation data is presented.

Site Contact: Dr. Chris Tinius, Asgrow Seed Company

Site of Release: Marion, AR

Date of Release: June, 1994

As requested in the supplemental conditions included with the approval of permit #94-090-02N, I am submitting a summary of the data collected from the field trial involving our Glufosinate tolerant soybeans at supervised by Dr. Chris Tinius, Asgrow Seed Co, at Marion, AR. The seed used in these studies were segregating populations crossed with homozygous transformed versions of Asgrow soybean varieties A5403 (known as W62) for breeding purposes.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Glufosinate tolerant soybeans were not unusually susceptible to any observable diseases or plant pests although there were no significant levels of disease present at that location. There was Soybean Cyst Nematodes present but the transgenic soybeans did not react any differently than non-transgenic soybeans.

Pesticide Tolerance: Ordinary soybean herbicides were applied. There was no apparent difference between Glufosinate tolerant and Glufosinate sensitive soybeans in their sensitivity to the other herbicides.

Date of Release Termination: October, 1994.

Inspection: Inspector was notified.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months and cultivated for 2 months post-harvest. The field will be rotated out of soybeans in 1995.

Means of Plant Disposition: The harvested seed was either stored on site or disposed of by returning it to the site and corporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil.

Site of Release: Oxford, IN

Date of Release: May, 1994

Site Contact: Dr. Hamer Paschal, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #94-090-02N, I am submitting a summary of the data collected from the field trial involving our Glufosinate tolerant soybeans at supervised by Dr. Hamer Paschal, Asgrow Seed Co, at Oxford, IN. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A3322 (known as W98) used for crossing purposes. There was 36 rows, 16 feet long of transgenic soybeans.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed.

Disease and Insect Susceptibility: The Glufosinate tolerant soybeans were not unusually susceptible to any observable disease. There was Brown Stem Rot and White Mold present but the transgenic soybeans did not appear to react any differently than non-transgenic soybeans.

Pesticide Tolerance: Ordinary soybean herbicides were applied. There was no apparent difference between Glufosinate tolerant and Glufosinate sensitive soybeans in their sensitivity to the other herbicides.

Date of Release Termination: October, 1994.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months and cultivated for 2 months post-harvest. It will be rotated out of soybeans in 1995.

Means of Plant Disposition: The harvested seed was either stored on site or disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil.

Site of Release: Stonington, IL

Date of Release: June, 1994

Site Contact: Dr. Craig Moots, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #94-090-02N, I am submitting a summary of the data collected from the field trial involving our Glufosinate tolerant soybeans at supervised by Dr. Craig Moots, Asgrow Seed Co, at Stonington, IL. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A5403 (known as W62) and A3322 (known as W98) as well as F4 progeny rows and 0.2 acre of bulk F6 increases.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed. segregation data of the F progeny rows for Glufosinate tolerance is presented in Table 1. Each row of F material was designated Resistant, Susceptible or Heterogeneous in its reaction to Glufosinate. The data did not fit the single dominant gene model, failing to conform to the expected genetic ratios.

The lack of fit may be due to two factors. In the F3 generation, the bulk plots were sprayed with Glufosinate. This uld have eliminated any homozygous susceptible lines, leaving no susceptible F rows. As Table 1 shows some populations had large numbers of susceptible rows. This aberration is likely due to incomplete spraying in the previous F3 generation with Glufosinate. The breeder confirmed that the F3 bulk Glufosinate soybean plots were close to susceptible corn plots. In their attempt to keep the Glufosinate off the susceptible corn, they probably did not completely eliminate the susceptible soybean plants, thus allowing for susceptible rows in the F generation.

A second problem with the results is that there is a much higher proportion of heterogeneous rows that would be expected. In the F generation, the rows were classified as heterogeneous if one or more plant appeared to be injured and dying due to its susceptibility to Glufosinate. It is possible that the sometimes sporadic plant death that was occurring was due to pathogens which were not widely identified, such as soil borne pathogens. If even a single plant in the row was showing signs of injury or death, the entire row was classified as heterogeneous. We are investigating this further.

Disease and Insect Susceptibility: The Glufosinate tolerant soybeans were not unusually susceptible to any observable diseases or plant pests although there were no significant levels of diseases or insects present at that location that year.

Pesticide Tolerance: Ordinary soybean herbicides were applied. There was no apparent difference between Glufosinate tolerant and Glufosinate sensitive soybeans in their sensitivity to the other herbicides.

Date of Release Termination: November, 1994.

Inspection: Inspector was notified.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months and cultivated for 2 months post-harvest. The field will be rotated out of soybeans in 1995.

Means of Plant Disposition: The harvested seed was either stored on site or disposed of by returning it to the site and incorporating it into the soil. The non-harvested transgenic residue was disposed of by incorporating it into the soil.

Table 1. Permit 94-090-02N Reaction of F4 Progeny rows to Glufosinate, Stonington, IL

Population	A3322 Glufosinate subline	Resistant	Susceptible	Heterogeneous	Total
Y913776	W98-7	28	2	114	114
Y913781	W98-7	12	0	23	35
Y913786	W98-7	32	3	61	96
Y913778	W98-7	35	4	105	144
Y913772	W98-7	7	15	26	48
Y913785	W98-7	21	4	70	95
Y913783	W98-3	14	0	38	52
Y913775	W98-3	5	1	90	96
Y913777	W98-3	0	22	26	48
Y913779	W98-3	14	0	35	49
Y913784	W98-3	9	27	61	97
Y913774	W98-7	10	7	79	96
Y913780	W98-7	33	4	101	138
Y913782	W98-7	33	1	94	128
Total		253	90	923	1266

	Resistant	Susceptible	Heterogeneous	Total
Observed F4 rows	253	90	923	1266
Expected F4 rows	759.6	0	506.4	
	(6Res)	(0Susc)	(4Hetero)	

$X^2 = 681$
$P <<< 0.005$

Site of Release: Ames, Iowa

Date of Release: June, 1994

Site Contact: Dr. Kevin Matson, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #94-090-02N, I am submitting a summary of the data collected from the field trial involving our Glufosinate tolerant soybeans at supervised by Dr Kevin Matson, Asgrow Seed Company at Ames, Iowa. The seed used in these studies were homozygous transformed sublines of Asgrow soybean varieties A3322 (known as W98). They were used for crossing purposes and for a weed control study.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed so far; the plots will continue to be monitored.

Disease and Insect Susceptibility: The Glufosinate tolerant soybeans were not unusually susceptible to any observable diseases or plant pests. There was Phytophthora Root Rot, Downy and Powdery Mildew present.

Pesticide Tolerance: Ordinary soybean herbicides were applied. There was no apparent difference between Glufosinate tolerant and Glufosinate sensitive soybeans in their sensitivity to the other herbicides.

Date of Release Termination: October, 1994.

Inspection: Inspectors were notified.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months and will continued to be monitored. The field will be rotated into corn in 1995. The weed control study was disked down before seeds were mature, August 5, 1994.

Means of Plant Disposition: The harvested seed from the crossing block was stored on site. The non-harvested transgenic residue from the crossing block was disposed of by incorporating it into the soil. The efficacy study was disked directly into the soil without allowing the seeds to reach maturity.

Site of Release: Janesville, WI

Date of Release: May, 1994

Site Contact: Dr. Andrew Nickell, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #94-090-02N, I am submitting a summary of the data collected from the field trial involving our Glufosinate tolerant soybeans at supervised by Dr Andrew Nickell, Asgrow Seed Company at Janesville, WI. The seed used in these studies were F7 generation or later transformed versions of Asgrow soybean varieties A3322 (known as W98). The trial was a small crossing block, 12 rows approximately 15 feet long.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed so far; the plots will continue to be monitored.

Disease and Insect Susceptibility: The Glufosinate tolerant soybeans were not unusually susceptible to any observable diseases or plant pests. There was Brown Stem Rot, Sclerotinia, Stem Canker, Bean Leaf Beetle, Soybean Looper and 2-spotted Spider Mites present at the site.

Pesticide Tolerance: Ordinary soybean herbicides were applied. There was no apparent difference between Glufosinate tolerant and Glufosinate sensitive soybeans in their sensitivity to the other herbicides.

Date of Release Termination: October, 1994.

Inspection: Regional Biotechnologist and BPEP Inspector inspected the field.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months and will continued to be monitored and volunteer plants destroyed. The field will be rotated out of soybeans in 1995.

Means of Plant Disposition: The harvested seed from the crossing block was stored on site. The non-harvested transgenic residue from the crossing block was disposed of by incorporating it into the soil.

Site of Release: Galena, MD

Date of Release: June, 1994

Site Contact: Bill Rhodes, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #94-090-02N, I am submitting a summary of the data collected from the field trial involving our Glufosinate tolerant soybeans at supervised by Bill Rhodes Asgrow Seed Company at Galena, MD. The seed used in these studies were transformed versions of Asgrow soybean varieties A3322 (known as W98) and A5403 (known as W62), used for a demonstration plot for Asgrow.

Phenotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed so far; the plots will continue to be monitored.

Disease and Insect Susceptibility: The Glufosinate tolerant soybeans were not unusually susceptible to any observable diseases or plant pests. There were no substantial diseases or insect pressures.

Pesticide Tolerance: Ordinary soybean herbicides were applied. There was no apparent difference between Glufosinate tolerant and Glufosinate sensitive soybeans in their sensitivity to the other herbicides. Glufosinate was not sprayed.

Date of Release Termination: Fall, 1994

Inspection: A Regional Biotechnologist and BPEP Inspector inspected the field.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months and will continued to be monitored. The field will be rotated out of soybeans in 1995. The demonstration trial was not harvested for seed but mowed down and incorporated into the soil.

Means of Plant Disposition: The transgenic residue from the demo was disposed of by incorporating it into the soil.

~UMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT: #94-131-01N

Permittee: Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road
Kalamazoo, MI 49002-1782; (616) 384-5531

Purpose: To evaluate efficacy and agronomics of glufosinate tolerant soybeans.

Results: Qualitative comparisons between glufosinate tolerant and non-transgenic soybeans.

Site of Release: McLean County, IL

Date of Release: May 20, 1994

Site Contact: Brian Freed, Asgrow Seed Company

As requested in the supplemental conditions included with the approval of permit #94-131-01N, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at McLean County, IL, supervised by Brian Freed, Asgrow Seed Co. The purpose of the trial was to test the agronomic performance of transgenic soybean plants under different cultural conditions and to obtain efficacy data for the herbicide IGNITE. The seed used in these studies were homozygous transformed versions of Asgrow soybean varieties A3322 (known as W98).

.henotypes: There were no unexpected phenotypes. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. The transgenic plants showed no phenotypic change which might allow them to become a weed. No unusual changes in overwintering habits were observed so far but the site will continue to be monitored.

Disease and Insect Susceptibility: The Ignite tolerant soybeans were not unusally susceptible to any observable diseases or plant pests although there were no significant levels of disease and insect pests present at that location.

Herbicide Tolerance: Dates of Ignite spraying were June 21 and July 1, 1994. Glyphosate was the only other pesticide applied before emergence.

Date of Release Termination: October, 1993.

Means of Containment: There are no wild or weedy relatives of soybeans in North America. Outcrossing in the crop itself is negligible. Thus there is almost no chance for the gene to be transferred through soybean pollen. To control volunteer soybean plants, the field was left unplanted for more than 5 months . The following year, the field will be rotated out of soybeans. The field will be monitored for a whole year and any volunteer soybean plants destroyed.

Means of Plant Disposition: The seed was not harvested. The entire trial was mowed and disked into the soil. No transgenic material was removed from the site.

Inspection: Inspectors were notified.

Final Report to the USDA - October 2, 1995

USDA Permit Number : 95-034-02N

Asgrow Permit Number: ASG13095.A

Locations: Isabela, Puerto Rico,
Union City, Tennessee

Responsible Researcher: Ms. Yolanda Otero-Ortiz (PR)
Mr. Leslie Lloyd (TN)

Acreage: Puerto Rico - 0.034 acres
Tennessee - 0.0073 acres

Dates of Release: 1/19/95 (Puerto Rico)
7/11/95 (Tennessee)
Machine planted - cleaned out

Date of Termination: June, 1995 (Puerto Rico)- hand harvested
November (estimated - Tennessee)- combine harvested

As requested in the supplemental conditions included with the approval of permit #95-034-02N, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Ms. Yolanda Otero and Mr. Leslie Lloyd, Asgrow Seed Co. The purpose of the trial was to increase seed, advance generations and demonstrate the the agronomic performance of transgenic soybean plants.

Means of Containment and Plant Disposition: All seed from Puerto Rico was shipped to the continental US in June, 1995. The entire plot wil be harvested for yield testing in Tennessee. After harvest, all seed will be returned to the field, incorporated by plowing or disking to eliminate volunteers. All residue in Puerto Rico was returned to the plot, incorporated by disking and plowing. The residue in Tennessee will remain at the plot site and will be incorporated by disking or plowing. Fields in both locations will be rotated out of soybeans and monitored for volunteer plants.

Disease and Insect Susceptibility: No changes is morphology, disease or insect resistance, or weediness was noticed between the transgenic and non-transgenic soybeans.

Results: Soybean lines in Puerto Rico were advanced a generation and multiplied to provide seed for the experiment in Tennessee. The trial in Tennessee was a herbicide trial, used to evaluate various herbicide treatments. Yield data will be collected and analyzed.

SUMMARY REPORT TO THE FIELD RELEASE OF TRANSGENIC SOYBEAN EXPRESSING RESISTANCE TO THE HERBICIDE GLUFOSINATE

DATE OF REPORT: November 8, 1995

NOTIFICATION NUMBERS:

95-069-01N	95-069-06N	95-069-11N
95-069-02N	95-069-07N	95-069-12N
95-069-03N	95-069-08N	95-079-02N
95-069-04N	95-069-09N	95-115-04N
95-064-05N	95-069-10N	95-135-04N
		95-142-02N

APPLICANT: Dr. Ed Pieters
AgrEvo USA Company
2711 Centerville Road
Wilmington, DE 19808

DATES OF RELEASE: April through August 1995

DATES OF TERMINATION: July through November 1995

SITES OF RELEASE (States/Number per State): Alabama/1, Arkansas/5, Florida/1, Georgia/4, Illinois/10, Indiana/8, Iowa/19, Kansas/2, Kentucky/2, Louisiana/2, Maryland/1, Michigan/2, Minnesota/4, Mississippi/4, Missouri/5 New Jersey/1, North Carolina/4 North Dakota/3, Nebraska/4, Ohio/7, Pennsylvania/3, South Carolina/1, South Dakota/2, Tennessee/4, Virginia/2, Wisconsin/4.

PURPOSE OF RELEASE

To evaluate weed control with glufosinate herbicide when applied to soybean plants containing the *bar* gene which confers resistance to glufosinate herbicide. The transgenic material was W98 derived from Asgrow 3222 variety maturity group 3 for midwest soybean production and W98 derived from Asgrow 5403 variety maturity group 5 for southern soybean production.

RESULTS

Glufosinate herbicide provided control of economically important weeds in soybean with no injury to the transgenic soybean plants.

OBSERVATIONS

The frequency of observations differed with each location. Each location was visited one or more times during the duration of the release. The area planted to transgenic soybean ranged from 0.2 to 10.0 acres per site. The transgenic soybean population was an average of 150,000 plants per acre.

Herbicide Tolerance: The transgenic soybean plants exhibited resistance to glufosinate herbicide. The transgenic soybean plants were also tolerant to other commercially used soybean herbicides that were used in the trials as standards. The nontransgenic soybean was severely injured by treatment with glufosinate.

Insect Susceptibility: The primary insect pests of soybean are green cloverworm, soybean loopers, stink bugs, and leafhoppers. There were no differences between transgenic and nontransgenic soybean.

Disease Susceptibility: Asgrow 3222 W98 Group 3 soybean are moderately resistant to brown stem rot, resistant to phytophthora, and susceptible to soybean cyst nematode. Asgrow 5403 W62 Group 5 soybean are susceptible to sudden death syndrome and phytophthora, moderately susceptible to frog eye, and resistant to soybean cyst nematode and stem canker. No deterioration of the resistant traits have been observed over the past four years. Casual observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybean.

Weather Related Conditions: The weather conditions were high moisture early in the season, followed by dryer than normal conditions during the mid season.

Physical Characteristics: The soybean plants were observed from emergence through maturity. No differences were observed between transgenic and nontransgenic soybean in emergence, seedling vigor, and stand establishment. Prior to glufosinate application no morphological differences were observed between the transgenic and non-transgenic plants. After glufosinate application, the transgenic plants continued to grow normally. The nontransgenic soybean was severely injured by glufosinate.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and nontransgenic pants.

MEANS OF PLANT DISPOSITION

The destruction of the plants differed by site and consisted of mechanical mowing, disking, land fill, and/or plowing.

TIME/METHODS OF MONITORING FOR VOLUNTEERS

Sites will be visited one or more times in the spring of 1996 when soil temperatures reach a level at which soybean emergence will be expected. The sites will be visually inspected for volunteer soybean plants. If any volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

NUMBER OF VOLUNTEERS OBSERVED/ACTION TAKEN

The number of volunteer soybean plants will be observed and recorded in 1996. All volunteer soybean plants will be destroyed by mechanical means, removed by hand, or destroyed with herbicides other than glufosinate.

Final Report to the USDA - October 6, 1995

USDA Permit Number : 95-122-03N

Asgrow Permit Number: ASG042695.A

Location: Galena, MD

Responsible Researcher: Mr. William Rhodes

Experiment Size: 57 plots, from 6 to 250 plants per plot

Dates of Release: June 15, 1995

Date of Termination: October - hand harvested

As requested in the supplemental conditions included with the approval of permit #95-122-03N, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Mr. Billy Rhodes, Asgrow Seed Co. The purposes of the trial was to increase seed, advance generations and study segregation ratios.

Means of Containment and Plant Disposition: All seed harvested will be stored under approved conditions or shipped under permit to other Asgrow stations. After harvest, all seed not used for further testing will be returned to the field, incorporated by plowing or disking to eliminate volunteers. All residue will be returned to the plot, incorporated by disking and plowing. The field will be rotated out of soybeans next year and monitored for volunteer plants.

Disease and Insect Susceptibility: No changes is morphology, disease or insect resistance, or weediness was noticed between the transgenic and non-transgenic soybeans.

Inspectors: Inspectors were notified before planting.

Results: Soybean lines were advanced a generation and multiplied to provide seed for future Asgrow breeding and testing. Segregation data on the various sublines was collected, as shown in Table 1. Expected ratios for these R1 plants was 3:1, Resistant:Susceptible. Deviations from the expected ratio can be explained by the small sample size as well as chimeric portions on the plant leading to seeds that were not resistant. Selections were made from the lines having the best phenotype as well as the closest segregation ratios. These lines will be studied further genetically to determine the stability of the gene.

Table 1. Segregation Data of Glufosinate Soybeans, Galena, MD
Summer, 1995.

	Obs Res	Sus	Total	Exp Res	Sus	X2
Total	757	469	1226	921	307	53140
A2704 L	119	33	152	114	38	50
A2704	134	91	225	168	56	2381
A5547 L	247	68	315	237	79	221
A5547	257	277	534	402	134	41474

SUMMARY REPORT - Field Release of Transgenic Soybeans with Glufosinate Tolerance

PERMIT:	#92-308-01
Permittee:	Ted Diedrick, Asgrow Seed Company, 2605 E. Kilgore Road Kalamazoo, MI 49002-1782; (616) 384-5531
Purpose:	To make crosses between Asgrow elite germplasm and glufosinate transgenic soybeans; to advance generations and increase material
Results:	No field trials were undertaken under this permit
Site of Intended Release:	Isabela, PR
Date of Release:	Not released
Site Contact:	Yolanda Otero-Ortiz, Asgrow Seed Company

As requested in the conditions included with the approval of permit #92-308-01, I am submitting a final report. Because we did not need to increase our stocks of Ignite tolerant soybeans, the field trials covered by this permit were never undertaken.