98-014-01p

AgrEvo

January 12, 1998

Mr. Michael A. Lidsky
USDA, APHIS, PPQ, BSS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Re: **Request for Extension of a Determination of Nonregulated Status forGlufosinate Resistant Soybean Transformation Events (96-068-1p)**

Dear Mr. Lidsky:

AgrEvo USA Company is submitting an Application for an Extension of the Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS) for Glufosinate Resistant Soybean (GRS) Events previously granted under 96-0681p. The new event is called **A5547-127.** This event was transformed in the same manner and using the same plasmid as were the events in the previous petition. The data submitted supports the contention that this event exhibits the same properties as the previously approved events. Termination reports from all relevant field trials are enclosed.

Enclosed are two copies of the petition extension. The enclosed request does not contain any confidential business information. Please contact me at (302) 892-3155 if you have any questions concerning our petition.

Best Regards,

Sally Van Wert

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

Enclosures (2)

A5547-127 soybean extension file

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1/14/98

Application for an Extension of the Determination of Nonregulated Status for Glufosinate Resistant Soybean Transformation Events (96-068-1p):

Event A5547-127

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

Submitted by:

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

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January 12, 1998

Contains No Confidential Business Information

Summary

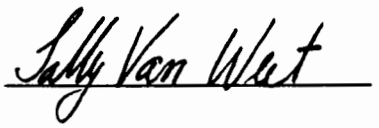
Event A5547-127 has been field tested by AgrEvo USA Company and Asgrow Seed Company since 1996 in the primary soybean growing regions of the southern United States. These tests have occurred at approximately 67 sites under field release authorizations granted by APHIS (USDA authorizations: 96-032-03N, 96-071-14N, 96-099-07N, 96-338-01N, 97-020-09N, 97-077-07N, 97-077-08N, 97-077-09N, 97-077-10N, 97-077-11N, 97-077-12N, 97-077-13N, 97-080-06N, 97-098-02N, 97-098-05N, 97-111-05N and 97-120-01N). Data collected from these trials, laboratory analyses, reports, and literature references presented herein demonstrate that Glufosinate Resistant Soybean (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 event A5547-127 has also been field tested in Argentina.

Primary transformation event A5547-127 has been self-pollinated and crossed with Asgrow's proprietary lines. The primary transformation event and its progeny are collectively referred to as GRS in this petition extension.

AgrEvo USA Company requests a determination from APHIS that GRS transformation event A5547-127 and any progeny derived from crosses of this event with traditional soybean varieties, and any progeny derived from crosses of this event 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

bla - ß-lactamase gene, ampicillin resistance gene
ELISA - enzyme linked immunosorbent assay
GA - glufosinate-ammonium
GRS - glufosinate resistant soybean
PAT - phosphinothricin acetyltransferase
pat - phosphinothricin acetyltransferase gene (origin *S. viridochromogenes*)
PCR - polymerase chain reaction

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Statement of Grounds for Nonregulated Status

I. Rationale for Submission of Request for Extension

There are no changes in rationale from the previously approved petition number 96-068-01p. The specific differences between Glufosinate Resistant Soybean Event (GRS) A5547-127 and the events in the previous petition are discussed in the appropriate sections. The new event to be considered under this extension is A5547-127. In notifications and termination reports event A5547-127 also has been referred to or designated as L5143-127, D5143-55-127, D5143-127 and B.

II. The Soybean Family

There are no changes from the previously approved petition submission.

III. The Transformation System and Plasmid Used

The GRS transformation event A5547-127 was transformed in the same manner and using the same plasmid (pB2/35AcK) as were GRS events A2704-12, A2704-21 and A5547-35 (see petition 96-068-01p for details). Asgrow Seed Company *Glycine max* cultivar A5547 was used for transformation. This is the same cultivar used for primary transformation event A5547-35. Prior to transformation the vector was digested with *Pvu*I to disrupt the coding sequence of the ß-lactamase (*bla*) gene, as was the case for events A2704-12 and A5547-127. As was the strategy for the previous GRS events, the commercialization strategy for A5547-127 is to use traditional crossing and breeding to transfer the glufosinate-ammonium (GA) resistance locus from the transformation event to a wide range of varieties with a wide range of maturities. Resistance to GA is conferred by expression of the phosphinothricin acetyltransferase (*pat*) gene.

IV. Genetic Characterization of Event A5547-127

A. Description, History and Mendelian Inheritance

Event A5547-127 was evaluated in the field in 1996 at 19 sites and in 1997 at 48 sites under authorizations granted by APHIS (USDA authorizations: 96-032-03N, 96-071-14N, 96-099-07N, 96-338-01N, 97-020-09N, 97-077-07N, 97-077-08N, 97-077-09N, 97-077-10N, 97-077-11N, 97-077-12N, 97-077-13N,97-080-06N, 97-098-02N, 97-098-05N, 97-111-05N and 97-120-01N). 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 inherited in a Mendelian fashion in A5547-127 homozygotes for a few generations. To begin the transfer of the *pat* gene from this transformation event to commercially viable material the original hemizygous transformed plant was self-pollinated. The GA resistant progeny were again self-pollinated. The resultant progeny were evaluated early in the 1996 growing season and resulted in R2 progeny rows segregating in a 1:2 fashion (entire rows resistant : partial rows resistant) with respect to glufosinate resistance (Table 1). The progeny from fully resistant rows were homozygous for the *pat* locus while those from partially resistant rows were heterogeneous with respect 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 in subsequent seasons. This was evaluated during the 1997 growing season and found to be true. The commercial lines were selected from these homozygous plants.

To confirm the expected Mendelian segregation of 3:1 for the *pat* locus, the progeny (R3) from the heterogeneous rows were planted in the later part of the 1996 growing season. Progeny from most rows segregated in a 3:1 fashion with respect to glufosinate resistance (Table 1). The reason for some rows not segregating as expected (rows 9, 14, 25, 29) is that some plants in those rows were destroyed by white grubs prior to data gathering. Not all rows were affected by the grub infestation which was spotty. That the grubs appeared to favor the sensitive plants may be a coincidence. This was not investigated further. No further testing of the heterogeneous rows was performed once the expected segregation ratio was confirmed in the majority of rows. None of the progeny from the heterogeneous rows were used to generate commercial lines.

Table 1. Segregation Data for Individuals and Rows of Progeny of Self-pollinated Event A5547-127 [a]

Comparison	Progeny[b]	Resistant Rows	Sensitive Rows	Expected Ratio	χ^{2c}
all rows	R2	10	21	1:2	0.0

Comparison	Progeny[b]	Resistant Plants	Sensitive Plants	Expected Ratio	χ^{2c}
row 1	R3	66	16	3:1	1.52
row 2	R3	141	36	3:1	1.97
row 3	R3	35	7	3:1	1.84
row 4	R3	50	11	3:1	1.46
row 7	R3	40	7	3:1	2.72
row 9	R3	73	13	3:1	4.8*
row 10	R3	43	8	3:1	2.51
row 11	R3	34	6	3:1	2.13
row 13	R3	34	8	3:1	1.02
row 14	R3	32	3	3:1	5.26*
row 16	R3	41	10	3:1	0.89
row 17	R3	36	10	3:1	0.40
row 21	R3	76	21	3:1	0.52
row 22	R3	47	8	3:1	3.37
row 23	R3	120	31	3:1	1.69
row 25	R3	141	22	3:1	11.68*
row 26	R3	143	53	3:1	0.44
row 27	R3	80	16	3:1	3.56
row 29	R3	119	25	3:1	4.48*
row 30	R3	148	46	3:1	0.23
row 31	R3	34	8	3:1	1.02

[b] Data taken from termination report 96-032-03N (See Appendix).

[b] R2 = segregation of entire versus partially resistant rows derived from resistant R2 plants; R3 = segregation of individual progeny from hemizygous R2 plant rows.

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

B. DNA Analysis of Event A5547-127

To determine the nature and number of *pat* and *bla* gene insertions which occur in transformation event A5547-127, Southern hybridization was used. 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. Event A5547-127 differs in the copy number and extend of integrated DNA from the events that were the subject of petition 96-068-01p.

In the experiments restriction digested genomic DNA from transgenic plants homozygous for the integrated DNA were run in parallel with a digested genomic DNA from a nontransgenic A5547 plant, supplemented with approximately 1 copy of digested transforming plasmid. After blotting and hybridization with *pat*- and *bla* probes the number of copies (intact and/or 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. The dilution of the transformation vector 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. The determination of the integrated copies is deduced from analysing all obtained Southern blot data.

Several aliquots of event A5547-127 DNA were digested with restriction enzymes. See Figures 1 and 2 to locate restriction sites in pB2/35SAck. 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 *pat* gene fragment (Figures 3 and 4), or with ^{32}P-labeled *bla* gene fragments (Figures 5 and 6). The primers used to generate the gene fragments are between 46 and 22 nucleotides in length and were primarily located internal to the genes (Figure 2). Lanes contain approximately 10 µg of restricted DNA. The amount of restricted pB2/35SAck in positive control lanes is equivalent to 1.0 copy of the plasmid integrated in 10 µg of soybean DNA. The probed membranes were visualized by autoradiography. The same membranes were striped and rehybridized with different probes. Electronic scans of the autoradiographs are presented in this document.

The primer pairs used to generate probes and their target sequence are listed in Table 2. The locations of the primers are shown in Figure 2. The hybridizing fragments expected and observed when using the *pat* or *bla* gene probes are listed in Table 3. The sizes of some hybridizing fragments can be predicted by the location of restriction enzyme cleavage sites internal to the inserted vector.

Table 2. Primer Pairs and Target Sequences

Target sequence	Primer-pair	Probe size (bp)
pat (577 bp)	MDB403-MDB404	577
bla (866 bp)	MDB402-VDS41	866
5' *bla* + vector (957bp PvuI fragment)	MDB439-MDB438	957
3' *bla* (462 bp)	MDB402-MDB435	462

Table 3. Observed and Expected[a] Hybridizing Fragments in Southern Blots of A5547-127 DNA Probed with the *pat* or *bla* Gene

Digest	***pat* probe**		**3' *bla* probe**		**' 5' *bla* + vector ' probe**		***bla* probe**
	observed size	**expected size**	**observed size**	**expected size**	**observed size**	**expected size**	**observed size**
EcoRI	**1329**	1329	**2400**	>1673	**2800**	>957	**2800** **2400**
SphI	**1850**	>1240	**> 15000**	>1624	**1850**	>957	**>15000** **1850**
NcoI	**10000**	>2996	**10000**	>2996	**2400**	>957	**10000** **2400**
NcoI-HindIII	**1374**	1374	**6000**	>1622	**1500**	>957	**6000** **1500**
HindIII	**2900**	>1497	**6000**	>1622	**2900**	>957	**6000** **2900**
BamHI	**484** **315**	484 315	**2650**	>1657	**6000**	>957	**6000** **2650**
EcoRV	**8000**	>2379 181	**8000**	>2379	**4850**	>957	**8000** **4850**
DraI	**2500**	>2615	**550**	>485	**750**	>750 >207	**750** **550**

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

Those hybridizing fragments whose sizes cannot be predicted result from cleavage in the integrated vector and in the adjacent plant DNA. A schematic summary drawing of the insert of soybean event A5547-127 is presented in Figure 7.

1. *pat* Gene

Soybean event A5547-127 genomic DNA digested with EcoRI and hybridized with the *pat* probe (577 bp) showed the expected internal 1329 bp fragment (Figure 3, lane 2). NcoI-HindIII digested DNA also showed the expected internal 1374 bp fragment (Figure 3, lane 5). With BamHI digested A5547-127 DNA both internal fragments of 484 bp and 315 bp were observed (Figure 4, lane 5). These results indicate that a complete *pat* cassette is integrated into soybean event A5547-127.

A 1850 bp fragment was observed when probing SphI digested A5547-127 DNA with the *pat* probe (Figure 3, lane 3). With HindIII digested DNA a 2900 bp fragment was observed (see Figure 3, lane 6 and Figure 4, lane 4). Both enzymes cut the transforming DNA downstream of the *pat* gene.

A 10000 bp fragment was observed when probing NcoI digested DNA (Figure 3, lane 4) with the *pat* probe and a 8000 bp fragment when probing EcoRV digested DNA (Figure 4, lane 6). Both enzymes cut the transforming DNA upstream of the *pat* gene. Hybridization results obtained with the SphI, HindIII, NcoI and EcoRV restriction enzymes indicate that only one copy of the *pat* gene is integrated into the genome of soybean event A5547-127.

DraI digested A5547-127 DNA showed a 2500 bp fragment when probed with the *pat* probe (Figure 4, lane 8). This size is slightly smaller then the minimum expected length of 2600 bp. This indicates that some of the vector sequences upstream of the EcoRI site (position 458) were lost upon integration or were rearranged.

The intensity of the *pat* bands in the A5547-127 digests which remove the *pat* cassette from the vector (Figure 3, lanes 2 and 5) is less than the signal intensity of lanes 13 and 14 (1 copy). However, all the Southern blot data obtained, when probing soybean event A5547-127 DNA with *pat* the pat probe, indicate that one and only one *pat* gene copy is integrated into the plant genomic DNA.

2. *bla* Gene

Three probes were used to analyze the integration of *bla* sequences into the genome of soybean event A5547-127 plants (Figure 2):

- *bla*: this probe (866 bp) is homologous to *bla* sequences from position +8 (bp 3869) until 13 nucleotides (bp 3003) beyond the stop codon.
- 3' *bla*: this probe (462 bp) is homologous to *bla* sequences from position +411 (bp 3465) until 13 nucleotides (bp 3003) beyond the stop codon. This probe basically targets the DraI/PvuI fragment (bp 2975 - bp 3460).
- 5' *bla* + vector: this probe (957 bp) is homologous to pB2/35SAcK sequences from position 272 until position 3494. This probe basically targets the 957bp PvuI fragment (from bp 341 until bp 3460). It thus contains 5' *bla* sequences (from position +1 until position +381) and pB2/35SAcK vector sequences.

Soybean event A5547-127 DNA digested with DraI and hybridized with the *bla* probe showed a 550 bp fragment and a weaker 750 bp fragment (Figure 6, lane 8). The hybridization of the *bla* probe with pB2/35SAcK EcoRI-DraI digested DNA showed the expected 692 bp and 864 bp fragments (Figure 6, lane 7). The hybridization signal obtained with the 864 bp fragments is also weaker as compared to the 692 bp fragment. This is expected as the overlap of the *bla* probe with this 864 bp fragment is only 202 bp long. This observation, together with hybridization data obtained with the 3' *bla* and the 5' *bla* probes (Southerns not shown), allowed us to conclude that the 3' *bla* sequences are localized on the 550 bp DraI fragment and that the 5' *bla* sequences are localized on the 750 bp DraI fragment. Both DraI fragments are smaller than expected when complete plasmid fragments are inserted.

Soybean event A5547-127 DNA digested with SphI and hybridized with the *bla* probe showed two fragments: a 1850 bp fragment and a fragment with a size >15 kb (Figure 5, lane 3). When hybridizing the blot with the 5' *bla* probe, only the 1850 bp fragment hybridized and with the 3' *bla* probe only the >15 kb fragment hybridized (Southerns not shown). A5547-127 DNA digested with HindIII and probed with the *bla* probe also showed two fragments: a 6000 bp fragment and a 2900 bp fragment (Figure 5, lane 6). The 2900 bp fragment hybridized with the 5' *bla* probe and the 6000 bp fragment hybridized with the 3' *bla* probe. Alignment of the autoradiographs of the *pat* and 5' *bla* hybridization's showed that the 2900 bp HindIII and the 1850 bp SphI fragments also hybridized with the *pat* probe. It is therefore concluded that the 5' *bla* sequences are integrated upstream of the *pat*-cassette. Upon integration of the PvuI fragment containing the 5' sequences of the *bla* gene, rearrangements have occurred. The presence of a DraI restriction site in the close vicinity of the EcoRI site and the observation that the DraI fragment hybridizing with the *pat* probe was some 100

bp too short, are indicative for this. The Southern blot analysis didn't allow the determination of the orientation of the inserted 5' *bla* sequences.

Soybean event A5447-127 DNA digested with NcoI and hybridized with the *bla* probe showed two fragments: a 10 kb fragment that also hybridizes to the 3' *bla* probe, and a 2400 bp fragment that also hybridized to the 5' *bla* probe (Figure 5, lane 5). A5547-127 DNA digested with EcoRV also showed two fragments: a 8000 bp fragment that also hybridized with the 3' *bla* probe and a 4850 bp fragment that also hybridized with the 5' *bla* probe (Figure 6, lane 6). Again, this is additional evidence that there is not an intact *bla* gene copy integrated into the genome. Alignment of the autoradiographs of the *pat* and 3' *bla* hybridization's showed that the 10 kb NcoI and the 8000 bp EcoRV fragments also hybridized with the *pat* probe. We therefore can conclude that the 3' *bla* sequences are integrated downstream of the *pat*-cassette.

The Southern blot hybridization results obtained with the *bla* probes indicate that only one copy of the 3' end of the *bla* gene and only one copy of the 5' end of the *bla* gene are integrated into the plant genome. Both integrated parts of the *bla* gene do not reconstitute an intact *bla* gene: the 5' *bla* sequences are integrated upstream of the *pat* gene and the 3' *bla* sequences are integrated downstream of the *pat* gene.

In summary, Southern blot analysis of soybean event A5547-127 indicates that only one copy of the *pat* gene cassette is integrated into the plant genome. Hybridization results obtained with the different *bla* probes indicate that one copy of the 5' *bla* sequences is integrated upstream of the *pat* gene, and that one copy of the 3' *bla* sequences is integrated downstream of the *pat* gene. The 5' and 3' *bla* sequences are linked to the *pat* gene but do not reconstitute an intact *bla* gene. The probes were specific to the introduced sequences in event A5547-127 since no hybridization was seen with nontransgenic soybean (see Figure 3 and 5, lane 11; Figure 4 and 6, lane 1). A schematic summary drawing of the insert of soybean event A5547-127 is presented in Figure 7.

C. Gene Expression in Event A5547-127

The content of phosphinothricin acetyltransferase (PAT) protein in the transformation event A5547-127 was determined in leaf tissue by Enzyme Linked Immunosorbent Assay (ELISA). A polyclonal antibody was used in the ELISA . It detects both inactive and intact PAT enzyme. Therefore, the enzyme detected may not be functional. To determine whether any of the *bla* gene fragments were expressed Northern analysis was performed on several tissues types from event A5547-127. These analyses show that the *bla* gene fragments are not expressed in A5547-127.

1. PAT Expression.

The PAT ELISA is a sandwich immunoassay in which PAT specific antibodies are used to coat the wells and to bind to the primary antibody/PAT complex . Samples consisting of transformant extracts, non-transformant extracts as controls, and pure PAT protein as a standard are added to the wells. Following incubation, during which time the PAT in the sample is captured by the bound antibodies, the unbound material is removed. The second PAT antibody is labeled with horseradish peroxidase and it binds to PAT protien captured by the first anitbody. When the appropriate substrate is added the enzyme-labeled antigen-antibody complex converts it to a blue color. The resultant color development is proportional to the concentration of PAT protein in each microwell. Three dilutions of each extract are tested and the value nearest to the midpoint of the standard curve is used to determine the PAT content. ELISA assays were performed on leaf tissue from 10 day old soybean seedlings. Results from the ELISA are shown in Table 4.

Table 4. PAT Content [a] in Leaves of A5547-127 as Detected by ELISA

Plant [b]	mg TEP [c] / g sample	µg PAT/ g sample	% PAT/TEP	% PAT/fresh weight (g/g)
A5547-NT	5.0	ND [d]	0.00	0.00
A5547-127	4.6	1.72	0.037	1.72 x 10-4

[a] Values reported are the average from two replicate extractions from two samples of 10 day-old seedling leaves.
[b] NT = nontransformed.
[c] TEP = total extractable protein
[d] ND = not detectable. Limit of detection is 0.004 µg PAT/g matrix.

2. *bla* Expression.

Transformation event A5547-127 contains one copy of the 5' *bla* sequences is integrated upstream of the *pat* gene, and that one copy of the 3' *bla* sequences is integrated downstream of the *pat* gene (see Section IV. B). No expression of the *bla* sequences is expected since this gene is under the control of bacterial expression signals and should only be expressed in bacteria. Nevertheless, cryptic gene expression in this event was analyzed by Northern analysis of RNA extracted from several tissues and hybridized with a single stranded RNA probe

homologous to the region between the T7 and SP6 promoters found on pB2/35SAck (Figure 2).

In the positive control lanes (Figure 8, lanes 3-8) a 1768 nucleotide transcript is expected to hybridize with the probe. This is the size of the *in vitro* synthesized sense RNA. Hybridization was observed in lanes 3 and 4 of the dilution series. The limit of detection for this experiment is 2 pg (Figure 8, lane 4). These results demonstrate that the Northern analysis was performed under conditions allowing hybridization to the target sequences. No positive signal could be detected in any of the tested tissues (seed, root, stem, leaf) from A5547-127 and negative controls (A5547-NT) (Figure 8, lanes 9-16).

Figure 1. Vector Map of pB2/35SAck

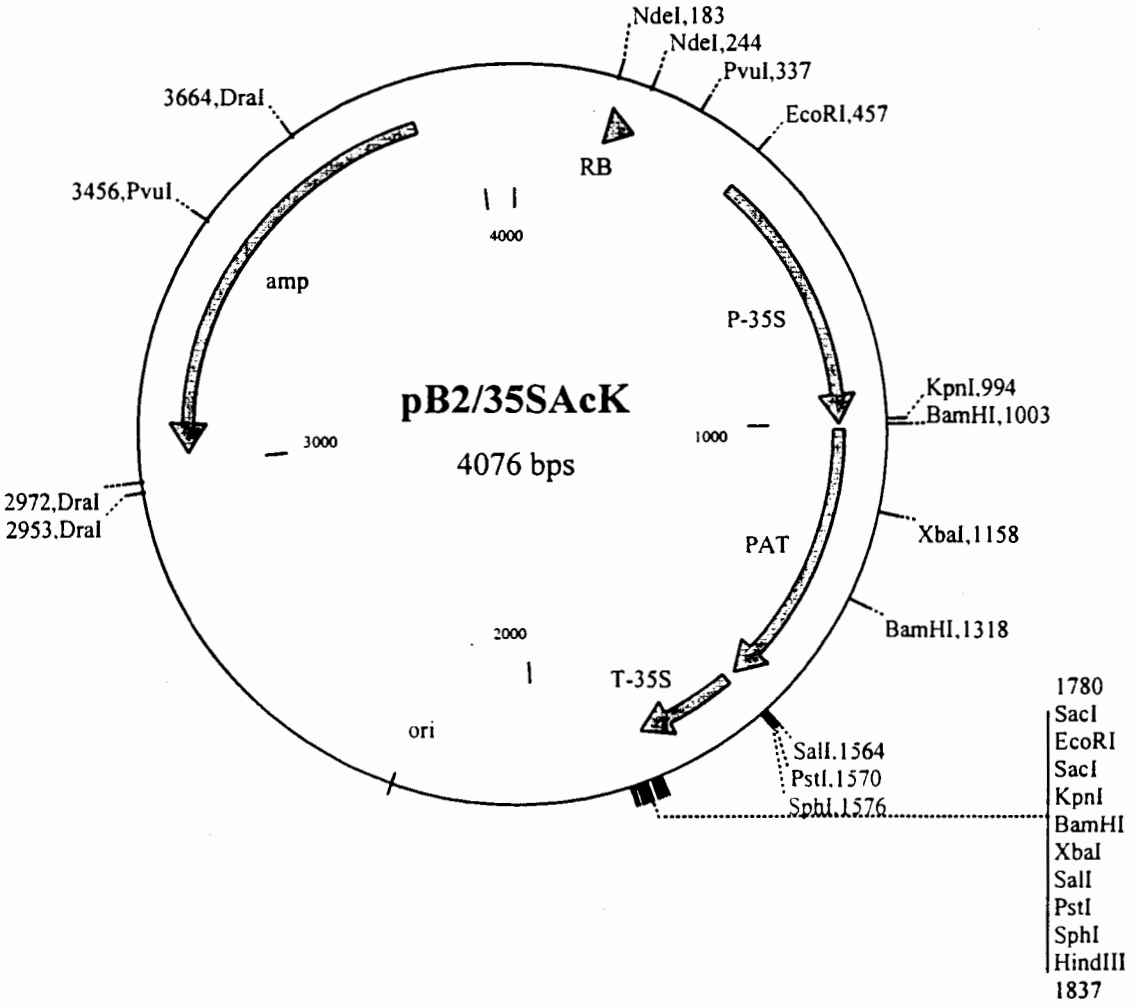


Figure 2. Location of primers on pB2/35SAcK

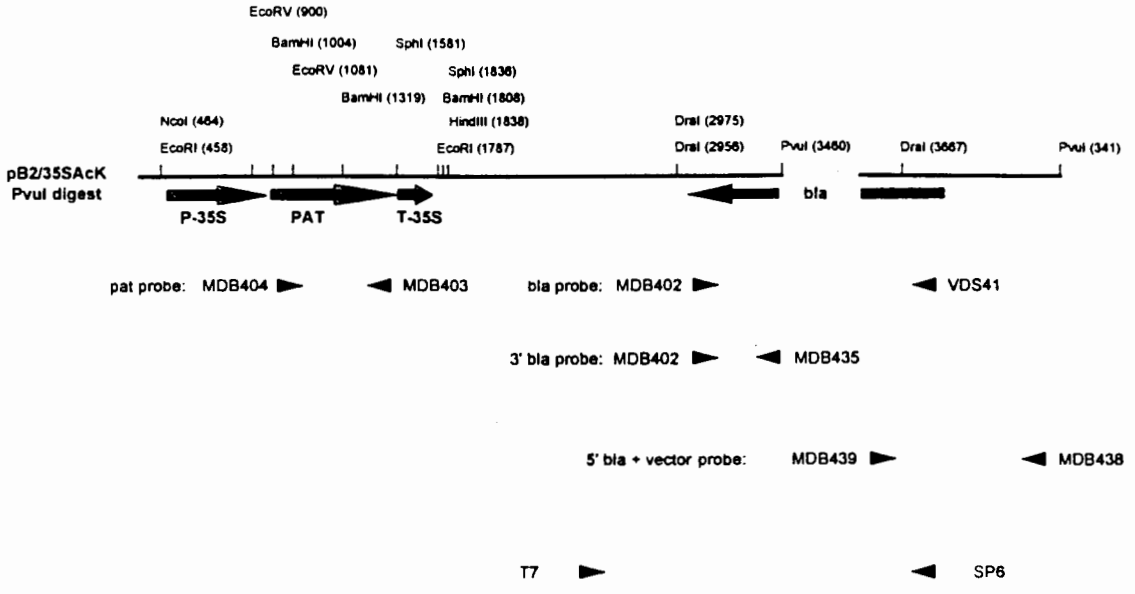


Figure 3. Southern Blot Analysis: Soybean Event A5547-127 - *pat* probe (blot 1). DNA was isolated from GRS event A5547-127 and the nontransgenic parent line A5547-NT. DNAs (10 μg) were digested with the indicated restriction enzymes. The *pat* fragment (577 bp)(see Figure 2) was used as probe. Lane 1. MW-marker. Lane 2. A5547-127: EcoRI digest. Lane 3. A5547-127: SphI digest. Lane 4. A5547-127: NcoI digest. Lane 5. A5547-127: NcoI/HindIII digest. Lane 6. A5547-127: HindIII digest. Lane 10. A5547-127: undigested. Lane 11. A5547 NT: HindIII digest. Lane12. MW-marker. Lane 13. pB2/35SAcK plasmid: EcoRI digest (supplemented with A5547-NT: HindIII digest). Lane 14. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with A5547-NT: HindIII digest). The amount of restricted pB2/35SAck in lanes 13 and 14 is equivalent to 1.0 copy of the plasmid integrated in 10 μg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

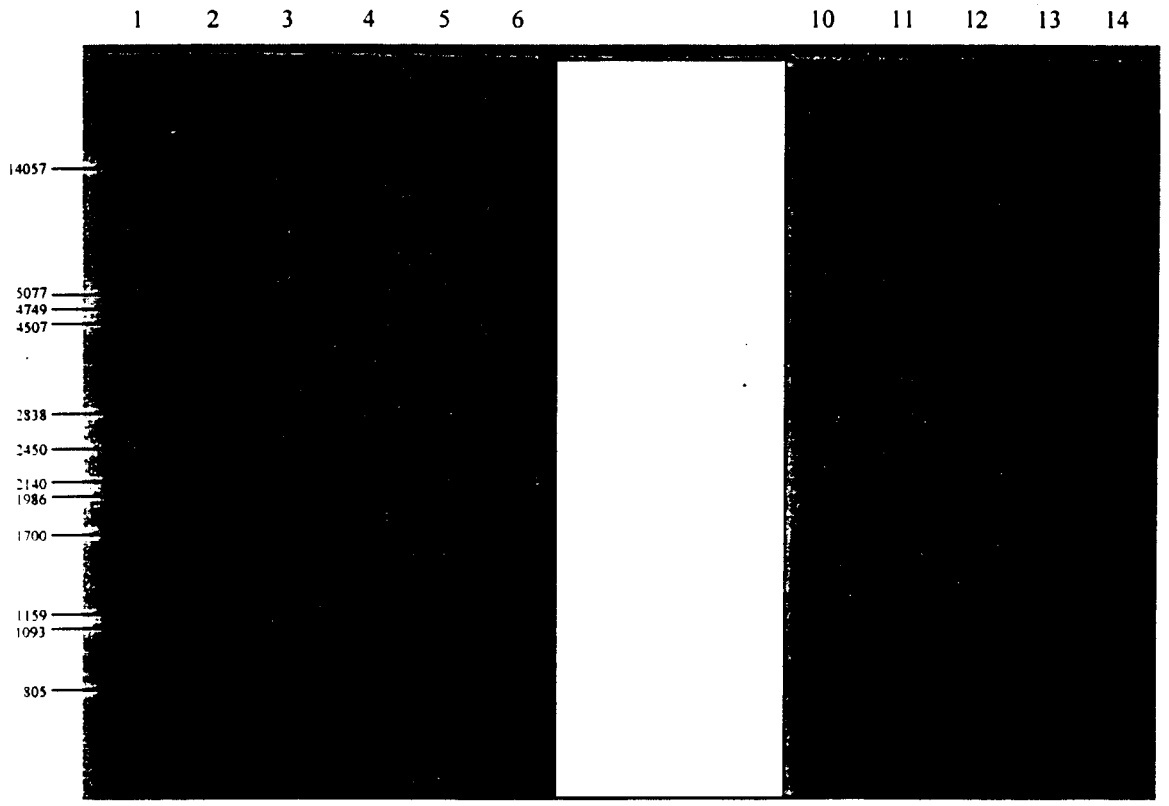


Figure 4. Southern Blot Analysis: Soybean Event A5547-127 - *pat* probe (blot 2). DNA was isolated from GRS event A5547-127 and the nontransgenic parent line A5547-NT. DNAs (10 μg) were digested with the indicated restriction enzymes. The *pat* fragment (577 bp)(see Figure 2) was used as probe. Lane 1. A5547-NT: undigested. Lane 2. MW-marker. Lane 3. A5547-127: undigested. Lane 4. A5547-127: HindIII digest. Lane 5. A5547-127: BamHI digest. Lane 6. A5547-127: EcoRV digest. Lane 7. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with A5547-NT: HindIII digest). Lane 8. A5547-127: DraI digest. The amount of restricted pB2/35SAcK in lane 7 is equivalent to 1.0 copy of the plasmid integrated in 10 μg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

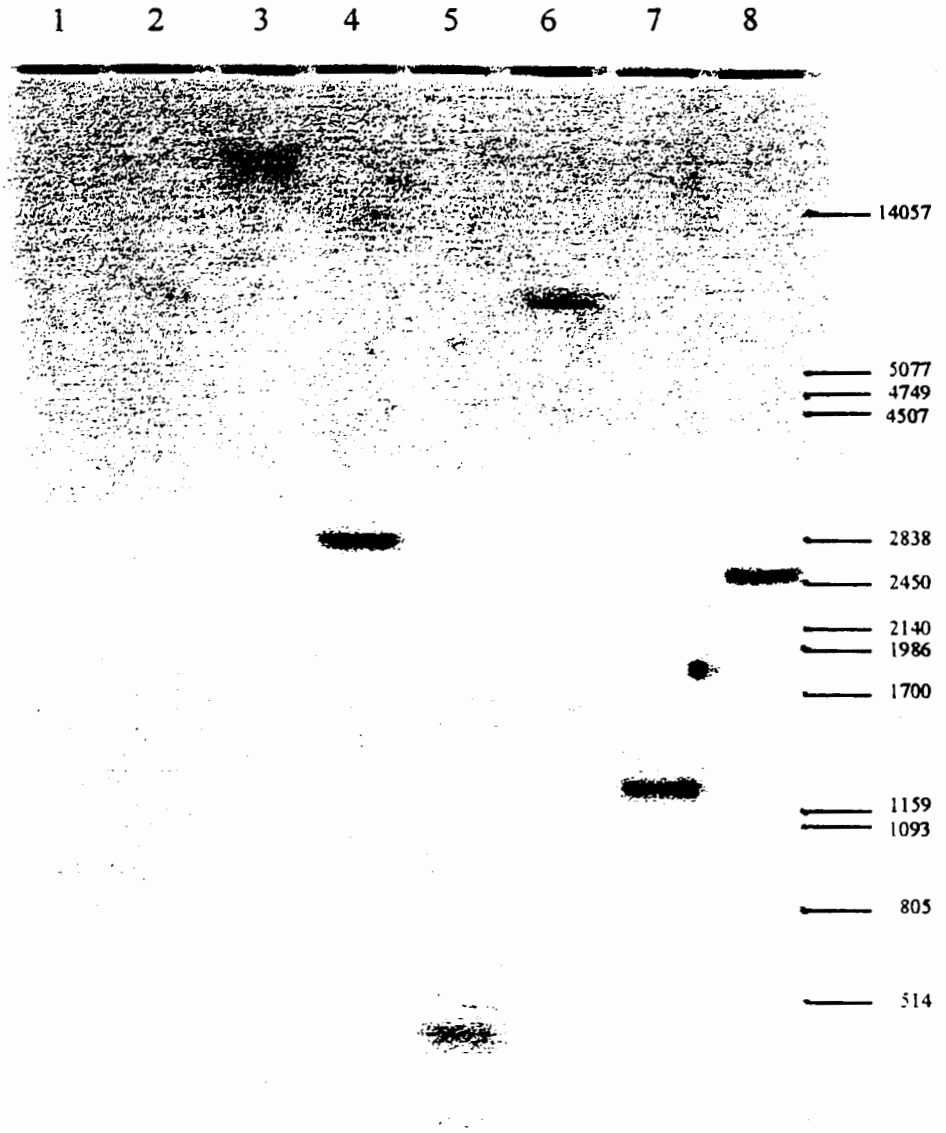


Figure 5. Southern Blot Analysis: Soybean Event A5547-127 - *bla* probe (blot 1). DNA was isolated from GRS event A5547-127 and the nontransgenic parent line A5547-NT. DNAs (10 µg) were digested with the indicated restriction enzymes. The *bla* fragment (866 bp)(see Figure 2) was used as probe. Lane 1. MW-marker. Lane 2. A5547-127: EcoRI digest. Lane 3. A5547-127: SphI digest. Lane 4. A5547-127: NcoI digest. Lane 5. A5547-127: NcoI/HindIII digest. Lane 6. A5547-127: HindIII digest. Lane 10. A5547-127: undigested. Lane 11. A5547 NT: HindIII digest. Lane12. MW-marker. Lane 13. pB2/35SAcK plasmid: EcoRI digest (supplemented with A5547-NT: HindIII digest). Lane 14. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with A5547-NT: HindIII digest). The amount of restricted pB2/35SAck in lanes 13 and 14 is equivalent to 1.0 copy of the plasmid integrated in 10 µg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

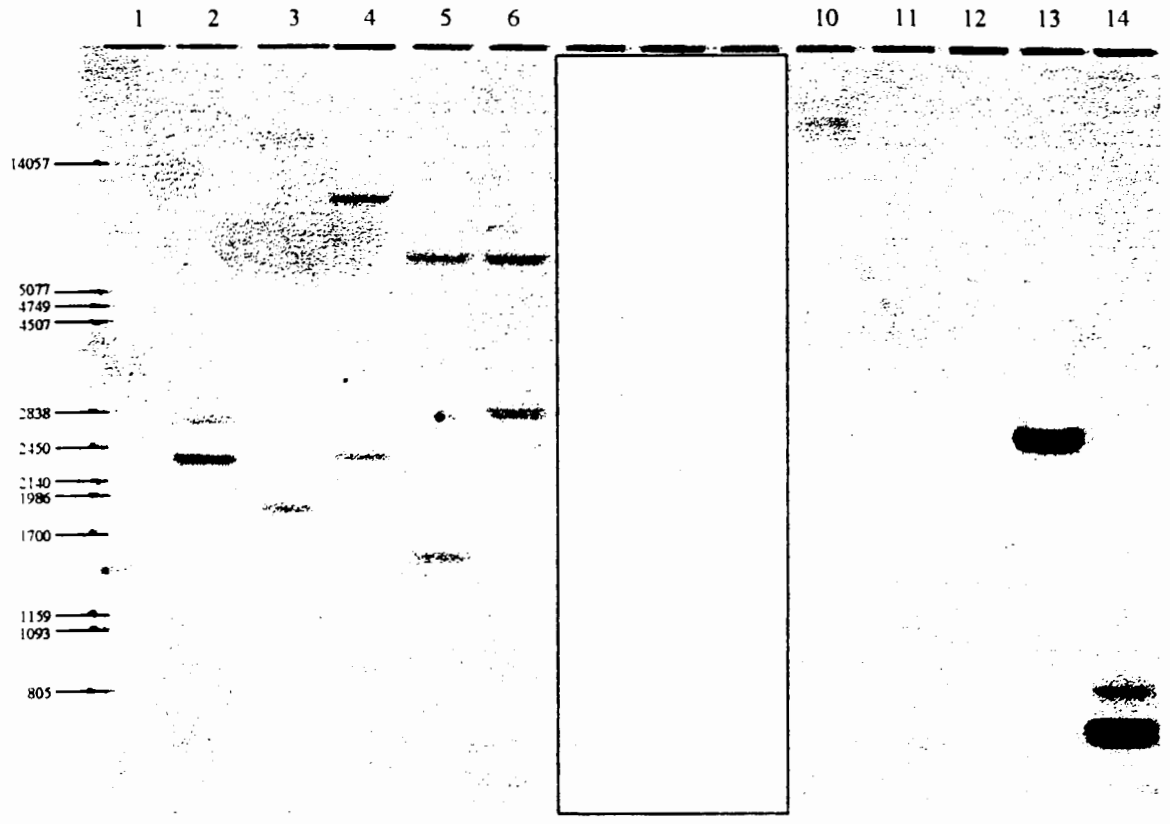


Figure 6. Southern Blot Analysis: Soybean Event A5547-127 - *bla* probe (blot 2). DNA was isolated from GRS event A5547-127 and the nontransgenic parent line A5547-NT. DNAs (10 μg) were digested with the indicated restriction enzymes. The *bla* fragment (866 bp)(see Figure 2) was used as probe. Lane 1. A5547-NT: undigested. Lane 2. MW-marker. Lane 3. A5547-127: undigested. Lane 4. A5547-127: HindIII digest. Lane 5. A5547-127: BamHI digest. Lane 6. A5547-127: EcoRV digest. Lane 7. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with A5547-NT: HindIII digest). Lane 8. A5547-127: DraI digest. The amount of restricted pB2/35SAcK in lane 7 is equivalent to 1.0 copy of the plasmid integrated in 10 μg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

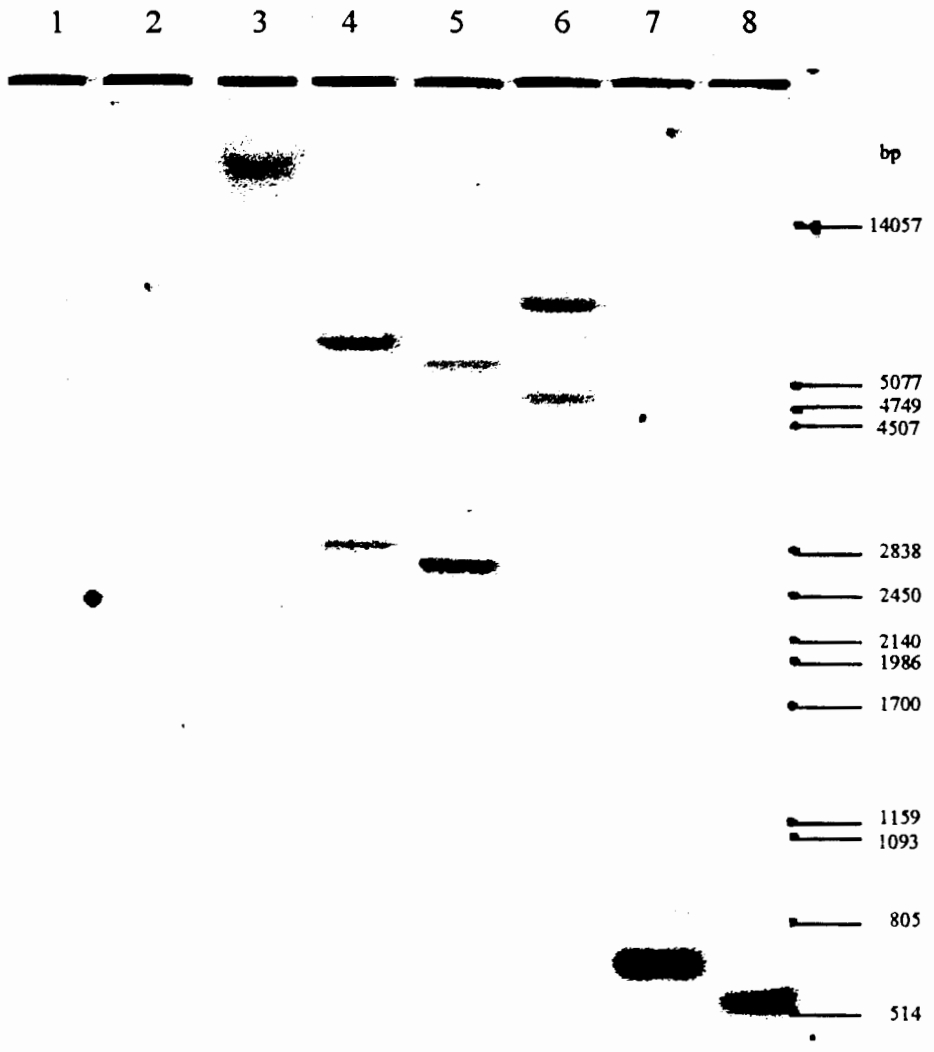


Figure 7. Schematic drawing of the insert in soybean event A5547-127.

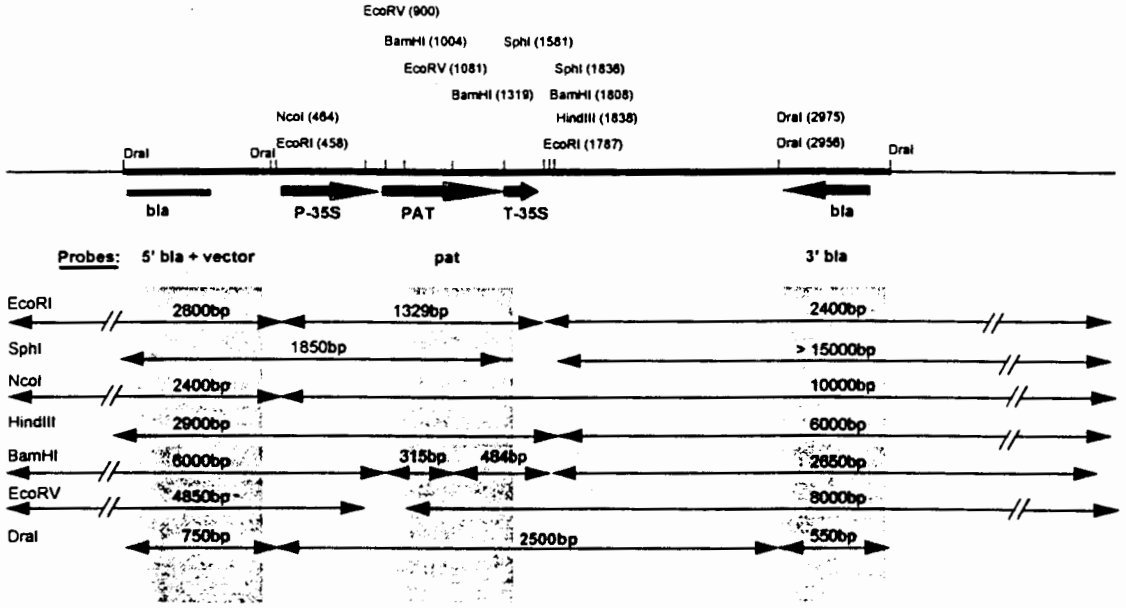
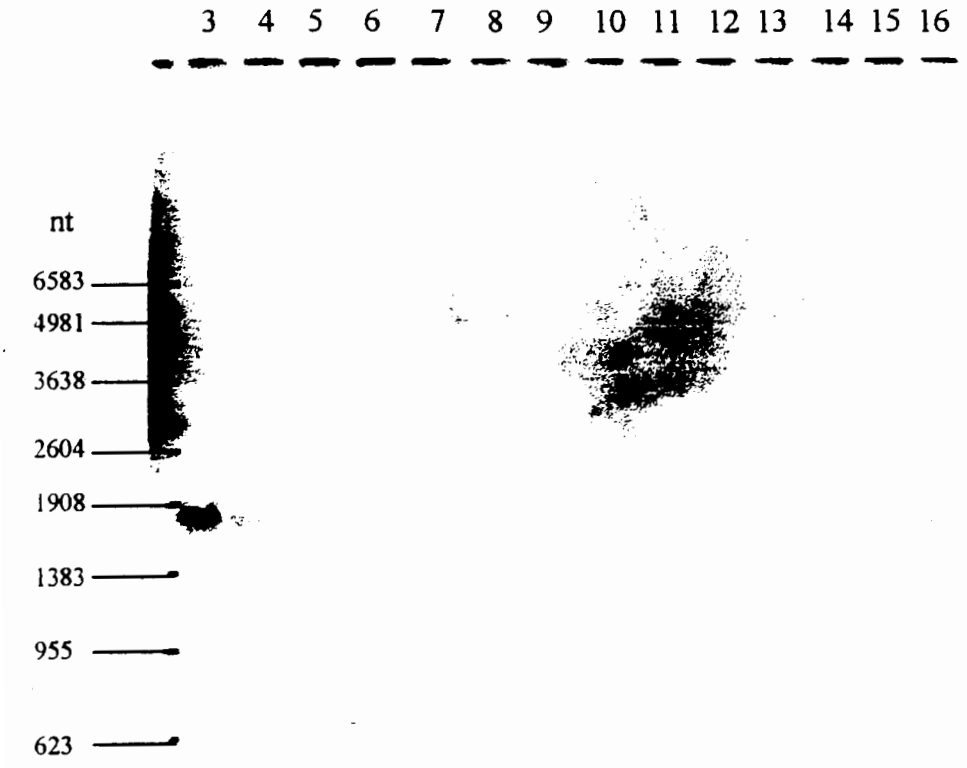


Figure 8. Northern Blot Analysis: Soybean Event A5547-127- *bla* probe. Lanes 3-8. *in vitro* synthesized sense *bla* RNA and A5547-NT leaf RNA. Lanes 9-16. RNA extracted from several tissues from GRS event A5547-127 and the nontransgenic parent line, A5547-NT. A single stranded RNA probe (1768 nt) homologous to the region between the T7 and SP6 promoters found on pB2/35SAck (see Figure 2) was used as probe. Lane 3. 4pg sense *bla* RNA. Lane 4. 2pg sense *bla* RNA. Lane 5. 1pg sense *bla* RNA. Lane 6. 0.5pg sense *bla* RNA. Lane 7. 0.25pg sense *bla* RNA. Lane 8. 0.1pg sense *bla* RNA. Lane 9. A5547-NT seed RNA. Lane 10. A5547-127 seed RNA. Lane 11. A5547-NT root RNA. Lane 12. A5547-127 root RNA. Lane 13. A5547-NT stem RNA. Lane 14. A5547-127 stem RNA. Lane 15. A5547-NT leaf RNA. 16. A5547-127 leaf RNA. RNA MW marker (G319, Promega Corporation) sizes given in nucelotides.



V. Agronomic Performance of Event A5547-127

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 96-032-03N in the Appendix). 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

Event A5547-127 was evaluated in the field in 1996 at 19 sites (Arkansas, Florida, Illinois, Indiana, Iowa, Maryland, Missouri, North Carolina, Puerto Rico and Wisconsin) and in 1997 at 48 sites (Alabama, Arkansas, Delaware, Florida, Georgia, Louisiana, Indiana, Maryland, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas, and Virginia) under authorizations granted by APHIS (USDA authorizations: 96-032-03N, 96-071-14N, 96-099-07N, 96-338-01N, 97-020-09N, 97-077-07N, 97-077-08N, 97-077-09N, 97-077-10N, 97-077-11N, 97-077-12N, 97-077-13N,97-080-06N, 97-098-02N, 97-098-05N, 97-111-05N and 97-120-01N). 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 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. The Appendix contains termination reports submitted to the USDA for the environmental releases that have been completed in the United States and Puerto Rico.

B. Agronomic, Disease and Pest Characteristics

Company researchers, university cooperators, and soybean breeders made visual observations of many agronomic traits of event A5547-127 including plant morphology, time of flowering, stand count, plant height, crop injury due to chemical application, maturity date, 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 1996 and 1997 growing seasons. For all agronomic information gathered, there were no significant 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 the Appendix). See Table 5 for certain quantitative agronomic data.

When the parent line, A5547, is propagated there is no selection and all seed is bulked together. Hence, individuals in the line are not all identical and the line contains a range of characteristics. In the breeding and selection process for commercial sublines of event A5547-127, yield was the main determinant for which sublines and rows were retained. These sublines were not bulked together with other sublines, but retained separate and self-pollinated. Sublines were further selected that bred "true" to type, with the result that the heterogeneity found in the parent line, A5547, was diminished in the population. During this "recurrent" selection process the breeder attempted to increase the agronomic value of the sublines; yield in this case. In the end, the selection process led to commercial material with good yield, and with a shorter height and earlier maturity than the nontransgenic parent line. The difference in height and

Table 5. Average Yield, Maturity, Height and Lodging of A5547-127 and Nontransgenic Parent Line in 1996 [a] and in 1997 [b, c]

Authorization Number	Plant [d]	Yield (bushels/acre)	Maturity (date)	Height (inches)	Lodging
96-032-03N	A5547-NT	68.3	Oct. . 8.0	33.0	2.3
	A5547-127	68.8	Oct. 4.0	28.8	2.3
	Std. Error	2.0	0.86	1.06	0.26
97-080-06N	A5547-NT	54.2	Oct. 20.4	37.6	2.7
	A5547-127	53.8	Oct. 18.9	36.2	2.9
	Std. Error	1.831	0.315	0.532	0.098
97-098-02N	A5547-NT	51.9	Oct. 23.5	38.5	2.9
	A5547-127	52.6	Oct. 22.1	37.0	3.1
	Std. Error	1.131	0.156	0.402	0.085

a Agronomic data collected from Arkansas and Maryland under authorization 96-032-03N. Values are the average of all rows of A5547-NT (parent line) from 2 sites, and the average of all rows from 6 sublines of A5547-127 from 2 sites.
b Agronomic data collected from Arkansas, Indiana, Maryland (2 sites), and North Carolina under authorization 97-080-06N. Values are the average of all rows of A5547-NT (parent line) or all rows of A5547-127 from all sites.
c Agronomic data collected from 3 sites in Maryland under authorization 97-098-02N. Values are the average of all rows of A5547-NT (parent line) or all rows of A5547-127 from all sites.
d NT = nontransformed. Std. Error = Standard Error of Means.

maturity are not considered significant by plant breeders. A difference of more than 3 days or 3-4 inches for maturity or height, respectively, must be observed for breeders to consider lines different. No significant differences between A5547-127 and its nontransgenic parent line (A5547-NT) were observed for yield and lodging.

Company researchers and cooperators made visual observations on several occasions for plant pathogenic organisms in trials containing event A5547-127 and its nontransgenic counterpart during the 1996 and 1997 growing seasons. Such observations revealed some minor pathogen infections but no infestations (see Appendix). Whenever pests were observed there were no differences in damage or populations found between GRS events and nontransgenic counterparts. The integration of vector DNA did not affect the inherent resistance of the parent cultivar to soybean cyst nematode. The beneficial insects - ladybugs and parasitic wasps - were observed in both transgenic and nontransgenic counterpart plots. As was found for the GRS events in the previously approved petition 96-068-01p, event A5547-127 did not influence susceptibility to disease or pest organisms in diverse genetic backgrounds and environments.

VI. Potential for Environmental Impact from Noncontained Use of Event A5547-127

There were no significant differences, apart from the intended change to glufosinate tolerance, demonstrated in field tests of event A5547-127 compared with the nontransgenic parent line. No morphological, beneficial organism, disease, or pest differences between A5547-127 and previously considered GRS events were noted. There is no reason to think cultivation of event A5547-127 and its progeny will have environmental effects different from cultivation of other GRS events which have already been considered by APHIS. No adverse consequences from the introduction of A5547-127 are expected.

VII. Statement of Grounds Unfavorable

No unfavorable information and data has been demonstrated for GRS Transformation Event A5547-127.

VIII. Appendix - USDA Field Trial Termination Reports

List of Release Authorizations

Authorization Number	States and Sites
96-032-03N	MD (1), PR (1)
96-071-14N	AR (1), IA (1), IL (1)
96-099-07N	AR (1), FL (1), NC (1)
96-338-01N	AR (2), IA (2), IL (2), IN (1), MO (1), PR (2), WI (1)
97-020-09N	not planted
97-077-07N	AL (3), FL (1), GA (1)
97-077-08N	MS (4)
97-077-09N	LA (3)
97-077-10N	TX (3)
97-077-11N	SC (2), VA (3)
97-077-12N	AR (4), MO (1), TN (3),
97-077-13N	NC (7)
97-080-06N	AR (1), IN (1), MD (2), NC (1)
97-098-02N	MD (3)
97-098-05N	not planted
97-111-05N	DE (1), MD (1)
97-120-01N	MO (3)

Asgrow Seed Company

Final Report to the USDA - January 22, 1997

USDA Permit Number	**96-032-03N**
Asgrow Permit Number:	AG013196.S
Applicant/ Responsible Person:	Dr Janet Nykaza, Asgrow Seed Company
Locations	Isabela, PR; Galena, MD
Responsible Researchers	Mr Juan Perez; Mr Bill Rhodes
Experiment Size	Isabela, PR: 3.2 acres Galena, MD: 0.2 acres
Dates of Release	Isabela, PR: May 31, 1996 Galena, MD: October 28, 1996
Date of Termination	Isabela, PR: Not harvested Galena, MD: November 6, 1996

As requested in the supplemental conditions included with the approval of permit **#96-032-03N**, I am submitting a preliminary summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Mr. Juan Perez and Mr. Bill Rhodes, Asgrow Seed Co. The trials in Puerto Rico will conclude in February and March. The purposes of the trial was to increase seed, advance generations, study segregation ratios, and do further hybridization.

Means of Containment and Plant Disposition: Machine planters were cleaned out after planting. A 20 foot fallow area was used to isolate the transgenic material. 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, and incorporated by plowing or disking to eliminate volunteers. All residue was either left at the plot or returned to the plot, and incorporated by disking and plowing. The field will be rotated out of soybeans next year and monitored for volunteer plants. The Puerto Rico plantings that are still in the field will follow the above protocol. We will notify you if there are any changes.

Disease and Insect Susceptibility: No changes is morphology, disease or insect resistance, or weediness was noticed between the transgenic and non-transgenic soybeans. White flies were noted at Galena, and in Puerto Rico common insects including White flies, leaf miners, and loopers were found, but but no differences in infestation between the transgenic and non-transgenic were noted in either location.

Inspectors: Inspectors were notified before planting in Puerto Rico.

Results: Soybean lines were advanced a generation and multiplied to provide seed for future Asgrow breeding and testing. Selections were made of the lines having the best phenotype. These lines will be studied further genetically to determine the stability of the gene. Segregation Ratios for various sublines are shown below.

L5143-125 demonstrated dominant single gene Mendelian ratios for both R1:2 plant rows as well as R2 individual plants.

L5143-127 demonstrated dominant single gene Mendelian ratios for both R2:3 plant rows as well as R3 individual plants.

Two L5143-0308 plants were inadvertently bulked at the R1 stage. As a result we were unable to get meaningful segregation data for this line. L5143-0309 produced 2 plants resistant to glufosinate. When the R2 plants were sprayed they were not segregating. This indicates that they are homozygous resistant. A summary of the R2 plant reaction for the two sublines is shown below. These lines in total do not fit a single gene model but we had a relatively small original sample size of 4 plants. We will reexamine segregation ratios in future generations for these sublines.

L5143-125 Segregation Data - R1:2 Plant Rows

Expected Ratio: 1:2

	#obs	#obs	Total	#exp	#exp	Chi	Probability	
Subline	homo	hetero	Rows	homo	hetero	Square		
L5143-125	11	19	30	10	20	0.14	0.75<P<0.5	ns

L5143-125 Segregation Data - R2 Plants

Expected Ratio: 3:1

	#obs	#obs	Total	#exp	#exp	Chi	Probability	
Subline	homo	hetero	Rows	homo	hetero	Square		
125-1	367	116	483	370	113	0.42	0.75<P<0.5	ns
125-4	344	107	451	338	113	0.42	0.75<P<0.5	ns
125-5	310	92	402	301	101	1.04	0.5<P<0.25	ns
125-7	249	80	329	247	82	0.07	0.9<P<0.75	ns
125-8	205	62	267	200	67	0.49	0.5<P<0.25	ns
125-9	239	72	311	233	78	0.60	0.5<P<0.25	ns
125-10	210	63	273	205	68	0.50	0.5<P<0.25	ns
125-11	258	74	332	249	83	1.30	0.5<P<0.25	ns
125-12	221	73	294	220	74	0.01	0.95<P<0.9	ns
125-14	201	65	266	199	67	0.07	0.9<P<0.75	ns
125-15	186	54	240	180	60	0.80	0.5<P<0.25	ns
125-16	180	55	235	176	59	0.35	0.75<P<0.5	ns
125-17	187	60	247	185	62	0.08	0.9<P<0.75	ns
125-19	151	47	198	148	50	0.22	0.75<P<0 5	ns
125-21	154	43	197	148	49	1.00	0.5<P<0 25	ns
125-23	162	52	214	160	54	0 09	0 9<P<0 75	ns
125-26	129	41	170	127	43	0.11	0.75<P<0 5	ns
125-27	97	39	136	102	34	0 98	0 5<P<0.25	ns
125-30	76	27	103	77	26	0 06	0 9<P<0 75	ns
Total	1714	529	2243	1682	561	2 42	P>0 995	ns

L5143-0308, 0309 Segregation Data - R2 Plants

Expected Ratio: 3:1

Subline		dead	alive	total	Chi Square	P
L5143-0308, -0309	obs	437	876	1313	48.08	P>0.005**
	exp	328	985			

L5143-127 Segregation Data - R2:3 Plant Rows

Expected Ratio: 1:2

	#obs	#obs	Total	#exp	#exp	Chi	Probability	
Subline	homo	hetero	Rows	homo	hetero	Square		
A5547-127	10	21	31	10	21	0	P<.995	ns

L5143-127 Segregation Data - R3 Plants

Expected Ratio: 3:1

	#obs	#obs	Total	#exp	#exp	Chi	Probability	
Subline	homo	hetero	Rows	homo	hetero	Square		
1	66	16	82	61	21	1.52	0.25<P<0.1	ns
2	141	36	177	133	44	1.97	0.25<P<0.1	ns
3	35	7	42	31	11	1.84	0.25<P<0.1	ns
4	50	11	61	46	15	1.46	0.25<P<0.1	ns
7	40	7	47	35	12	2.72	.1<P<0.05	ns
9	73	13	86	64	22	4.80	.050<P<0.025	ns
10	43	8	51	38	13	2.51	0.25<P<0.1	ns
11	34	6	40	30	10	2.13	0.25<P<0.1	ns
13	34	8	42	31	11	1.02	.5<P<0.25	ns
14	32	3	35	26	9	5.26	.025<P<0.01	ns
16	41	10	51	38	13	0.89	.5<P<0.25	ns
17	36	10	46	34	12	0.40	0.75<P<0.5	ns
21	76	21	97	73	24	0.52	.5<P<0.25	ns
22	47	8	55	41	14	3.37	.1<P<0.05	ns
23	120	31	151	113	38	1.69	0.25<P<0.1	ns
25	141	22	163	122	41	11.68	P>0.005	ns
26	143	53	196	147	49	0.44	0.75<P<0.5	ns
27	80	16	96	72	24	3.56	.1<P<0.05	ns
29	119	25	144	108	36	4.48	.050<P<0.025	ns
30	148	46	194	145	49	0.23	0.75<P<0.5	ns
31	34	8	42	31	11	1.02	.5<P<0.25	ns
						53.51	0.1<P<0.05	ns

Asgrow Seed Company

Final Report to the USDA - January 9, 1997

USDA Permit Number	**96-071-14N**
Asgrow Permit Number	AG030796.S
Applicant/ Responsible Person:	Dr. Janet Nykaza, Asgrow Seed Company
Locations:	New Baden, IL, Conrad, IA, Marion, AK
Responsible Researchers:	Mr. Jack Phillips, Mr. Tom Freed, Dr. Chris Tinius
Experiment Size:	New Baden: <0.5 acre Conrad: 400 ft^2 Marion: <0.5 acre
Dates of Release:	New Baden: May 23- June 17 Conrad: May 22 Marion: June 7
Date of Termination:	New Baden: October 2: Machine and hand harvested Conrad: September 3: Hand harvested Marion: October 24: Machine and hand harvested

As requested in the supplemental conditions included with the approval of permit **#96-071-14N**, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans at supervised by Mr. Jack Phillips, Mr. Tom Freed and Dr. Chris Tinius, all of Asgrow Seed Co. The purposes of the trial was to increase seed, advance generations, evaluate agronomic characteristics, and do further hybridization.

Means of Containment and Plant Disposition: Machine planters were cleaned out after planting. A 20 foot fallow area was used to isolate the transgenic material at New Baden and Conrad. At Marion, a 20 foot buffer of soybeans was used to isolate the trial. These plants were destroyed at the end of the trial and directly tilled into the soil. 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, and incorporated by plowing or disking to eliminate volunteers. All residue was either left at the plot or returned to the plot, and incorporated by disking and plowing. The fields will be rotated out of soybeans next year and continuously monitored for volunteer plants. At Conrad, the 4 transgenic plants were hand harvested before dry down so that there should be no volunteer seed remaining.

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 were advanced a generation and multiplied to provide seed.

for future Asgrow breeding and testing. Selections were made of the lines having the best phenotype. These lines will be studied further genetically to determine the inheritance of the gene.

A summary of agronomic data, collected at Marion, AK, as well as Galena, MD, under another notification, 96-032-03N, is shown in the table below:

Agronomic Data - Maturity Group V Liberty Link™ Variety - A5547 and L5143-127 -Marion, AK and Galena, MD

Variety	Yield (Bu/A)	Maturity	Height (In.)	Lodging
A5547	68.3	Oct. 8.0	33.0	2.3
L5143-127	70.1	Oct. 3.3	30.3	2.5
St. Error	2.0	0.86	1.06	0.26

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

DATE OF REPORT: October 15, 1997

NOTIFICATION NUMBER: 96-099-07N

APPLICANT: Dr. Sally Van Wert
AgrEvo USA Company
2711 Centerville Road
Wilmington, DE 19808

DATES OF RELEASE: April through August 1996

DATES OF TERMINATION: July through November 1996

SITES OF RELEASE (States/Number per State): Arkansas/1, Florida/1, Illinois/1, Iowa/1, North Carolina/1, Nebraska/1

PURPOSE OF RELEASE

To evaluate weed control with glufosinate herbicide when applied to soybean plants containing the *pat* gene which confers resistance to glufosinate herbicide. The transgenic material was A2704-12 and A2704-21 derived from Asgrow A2704 variety maturity group 2 and A5547-127 and A55547-35 derived from Asgrow 5547 variety maturity group 5 for southern soybean production.

A2704-12, A2704-21 and A5547-35 received non-regulated status 28 June 1996 (Docket No. 96-019-2). Therefore, this report only provides results for A5547-127 grown in Arkansas, Florida and North Carolina.

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 0.01 to 0.1 acres per site. The transgenic soybean population was an average of 150,000 plants per acre

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

Disease Susceptibility: Asgrow 5547-127 Group 5 soybean has tolerance to many leaf and stem diseases, and resistance to soybean cyst nematode races 3 and 14. No deterioration of the resistant traits were observed. Casual observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybean.

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.

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 were visited one or more times in the spring of 1997 when soil temperatures reached a level at which soybean emergence was expected. The sites were visually inspected for volunteer soybean plants. No volunteers were observed.

NUMBER OF VOLUNTEERS OBSERVED/ACTION TAKEN

No volunteers were observed.

Asgrow Seed Company

Final Report to the USDA December 15, 1997

USDA Permit Number: 96-338-01N

Asgrow Permit Number: AG961203S

Applicant/Responsible Person: Mr. Donald Steffen, Asgrow Seed Company

Locations: Isabela, PR; Santa Isabel, PR; Marion, AR; Oxford, IN; Janesville, WI; New Baden, IL; Matthews, MO; Marion, AR; Williams, IA; Atlantic, IA; and Ridgeway, IL.

The following locations were listed on the Field Release Notification, but were never planted (released): Schoolcraft, MI; Ames, IA; Redwood Falls, MN; Stonington, IL; Conrad, IA; Perry, IA; Tuscola, IL; Deschler, OH; Lexington, IL; Mapleton, MN; and, York, NE.

Responsible Researchers: Mr. Juan Perez-Real, Mr. Felix Heredia, Dr. Bruce Luzzi; Dr. Hamer Paschal; Dr. Andrew Nickell; Mr. Jack Phillips; Mr. Will Winslow; Mr. Larry Ganann;Mr. Brian Meese; Mr. Mark Wuebker; and, Mr. Mark Buettner.

Experiment Size:

Isabela, PR:	2.9 acres
Santa Isabel, PR:	8.0 acres
Marion, AR:	<0.1 acres
Oxford, IN:	<0.1 acres
Janesville, WI:	<0.1 acres
New Baden, IL:	<0.1 acres
Matthews, MO:	<0.1 acres
Marion, AR:	<0.5 acres
Williams, IA:	<0.5 acres
Atlantic, IA:	<0.5 acres
Ridgeway, IL:	<0.1 acres

Dates of Release:

Isabela, PR:	Feb. 17
Santa Isabel, PR:	Feb. 17
Marion, AR:	June 3
Oxford, IN:	June 19
Janesville, WI:	May [illegible]
New Baden, IL:	May 23-[illegible]
Matthews, MO:	May 2[illegible]
Marion, AR:	June 3
Williams, IA:	May [illegible]
Atlantic, IA:	May [illegible]
Ridgeway, IL:	[illegible]

Dates of Termination:

Isabela, PR:	May 8
Santa Isabel, PR:	May 8
Marion, AR:	Nov 17
Oxford, IN:	Nov.20
Janesville, WI:	Oct.19
New Baden, IL:	Oct. 30
Matthews, MO:	Oct. 18
Marion, AR:	Nov 19
Williams, LA:	Oct. 6
Atlantic, LA:	Sept. 29
Ridgeway, IL:	Oct. 31

As requested in the supplemental conditions included with the approval of permit #96-338-01N, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans supervised by Mr. Juan Perez-Real, Mr. Felix Heredia, Dr. Bruce Luzzi, Dr. Hamer Paschal, Dr. Andrew Nickell, Mr. Jack Phillips, Mr. Will Winslow, Mr. Larry Ganann, Mr. Brian Meese, Mr. Mark Wuebker, and Mr. Mark Buettner, all of Asgrow Seed Company. The purposes of the trial was to increase seed, advance generations, evaluate agronomic characteristics, assess weed control, and do further hybridization.

Means of Containment and Plant Disposition: Machine planters were cleaned out after planting. A 20 foot buffer of soybeans was used to isolate the transgenic material at all locations. These plants were destroyed at the end of the trial and directly tilled into the soil. All seed harvested will be stored under approved conditions or shipped under permit to other Asgrow stations. After harvest, seed not used for further testing will be returned to the field, and incorporated by plowing or disking to eliminate volunteers. All residue was either left at the plot or returned to the plot, and incorporated by plowing or disking. The fields will be rotated out of soybeans next year and continuously monitored for volunteer plants.

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

Results: Soybean lines were advanced a generation and multiplied to provide seed for future Asgrow breeding and testing. Selections were made of the lines having the best phenotype. No yield or harvest data were collected at these sites.

Asgrow Seed Company

Final Report to the USDA -- December 15, 1997

USDA Permit Number:	97-020-09N
Asgrow Permit Number:	AG970118.s
Applicant/Responsible Person:	Mr. Donald Steffen, Asgrow Seed Company
Locations:	The following locations were listed on the Field Release Notification, but were never planted (released): Santa Isabel, PR, Los Indios, TX, Matthews, MO, and Perry, LA.
Responsible Researchers:	Mr. Felix Heredia, Mr.John Sparks, Mr. Will Winslow, and Mr. Paul Brezina.
Experiment Size:	No field releases (plantings) were performed
Dates of Release:	No field releases (plantings) were performed
Dates of Termination:	No field releases (plantings) were performed

As requested in the supplemental conditions included with the approval of permit # 97-020-09N, I am submitting a summary of the data collected from the field trial involving our [illegible] soybeans supervised by Mr. Felix Heredia, Mr.John Sparks, Mr. Will Winslow, and Mr. Paul Brezina., all of Asgrow Seed Company. The purposes of the trial was to increase seed, advance generations, evaluate agronomic characteristics, assess weed control, and do further hybridization.

Means of Containment and Plant Disposition: No field releases (plantings) were performed.

Disease and Insect Susceptibility: No field releases (plantings) were performed.

Results: No field releases (plantings) were performed.

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

DATE OF REPORT: October 15, 1997

NOTIFICATION NUMBERS: 97-077-07N through 97-077-13N, 97-111-05N

APPLICANT: Dr. Sally Van Wert
AgrEvo USA Company
2711 Centerville Road
Wilmington, DE 19808

DATES OF RELEASE: April through August 1997

DATES OF TERMINATION: July through November 1997

SITES OF RELEASE (States/Number per State): Alabama/3, Arkansas/4, Delaware/1, Florida/1, Georgia/5, Louisiana/3, Maryland/1, Mississippi/4, Missouri/1, North Carolina/7, South Carolina/2, Tennessee/3, Texas/3, Virginia/3

PURPOSE OF RELEASE

To evaluate weed control with glufosinate herbicide when applied to soybean plants containing the *pat* gene which confers resistance to glufosinate herbicide. The transgenic material was A5547-127 derived from Asgrow 5547 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 0.01 to 0.1 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 severly 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 in pest infestations between transgenic and nontransgenic soybean.

Disease Susceptibility: Asgrow 5547-127 Group 5 soybean has tolerance to many leaf and stem diseases, and resistance to soybean cyst nematode races 3 and 14. No deterioration of the resistant traits were observed. Casual observations throughout the growing season did not note any disease infestations on either transgenic or nontransgenic soybean.

Beneficial Insects: The following beneficial insects were observed in plots - ladybugs and parasitic wasps. No differences in numbers were observed between transgenic and nontransgenic plots. Treatment with glufosinate also did not diminish beneficial populations.

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. Some harvesting is still underway, however, all residual plant material will be disposed of as stated. Some seed from sites will be shipped under movement authorizations for use in further studies.

TIME/METHODS OF MONITORING FOR VOLUNTEERS

Sites will be visited one or more times in the spring of 1998 when soil temperatures reached 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 1998. All volunteer soybean plants will be destroyed by mechanical means, removed by hand, or destroyed with herbicides other than glufosinate.

Asgrow Seed Company

Final Report to the USDA -- December 15, 1997

USDA Permit Number:	97-080-06N	
Asgrow Permit Number:	AS032297	
Applicant/Responsible Person:	Mr. Donald Steffen, Asgrow Seed Company	
Locations:	Galena, MD; Queenstown, MD; Kinston, NC; Newburgh, IN; and, Stuttgart, AR.	
Responsible Researchers:	Mr. Billy Rhodes, Mr. Rick Dougherty Mr. Mark Buettner, and Dr. Edward Brown	
Experiment Size:	Galena, MD:	0.8 acres
	Queenstown, MD:	0.1 acres
	Kinston, NC:	0.1 acres
	Newburgh, IN:	0.1 acres
	Stuttgart, AR:	0.1 acres
Dates of Release:	Galena, MD:	May 30
	Queenstown, MD:	June 7
	Kinston, NC:	May 23
	Newburgh, IN:	June 20
	Stuttgart, AR:	June 9
Dates of Termination:	Galena, MD:	Nov. 13
	Queenstown, MD:	Nov. 15
	Kinston, NC:	Oct. 18
	Newburgh, IN:	Oct. 24
	Stuttgart, AR:	Nov. 17

As requested in the supplemental conditions included with the approval of permit # [illegible], I am submitting a summary of the data collected from the field trial involving our [illegible] soybeans supervised by Mr. Billy Rhodes of Asgrow Seed Company. The purpose of the trial was to increase seed, advance generations, and evaluate agronomic characteristics.

Means of Containment and Plant Disposition: Machine planters were [illegible] planting. A 20 foot buffer of soybeans was used to isolate the trial [illegible]. These plants were destroyed at the end of the trial and directly [illegible] harvested will be stored under approved conditions or shipped under [illegible] stations. After harvest, seed not used for further testing will be returned [illegible].

incorporated by plowing or disking to eliminate volunteers. All residue was either left at the plot or returned to the plot, and incorporated by plowing or disking. The fields will be rotated out of soybeans next year and continuously monitored for volunteer plants.

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

Results: Soybean lines were advanced a generation and multiplied to provide seed for future Asgrow breeding and testing. Selections were made of the lines having the best phenotype

Variety	Yield (bu/a)	Maturity	Height (in.)	Lodging
A5547	54.2	Oct. 20.4	37.6	2.7
L5143-127	53.8	Oct. 18.9	36.2	2.9
Std. Error	1.831	0.315	0.532	0.095

Asgrow Seed Company

Final Report to the USDA -- December 15, 1997

USDA Permit Number:	97-098-02N	
Asgrow Permit Number:	AS040797	
Applicant/Responsible Person:	Mr. Donald Steffen, Asgrow Seed Company	
Locations:	Queenstown, MD, Eden, MD, and Hebron, MD	
Responsible Researchers:	Mr. Billy Rhodes	
Experiment Size:	Queenstown, MD:	0.1 acres
	Eden, MD:	0.1 acres
	Hebron, MD:	0.1 acres
Dates of Release:	Queenstown, MD:	June 7
	Eden, MD:	May 20
	Hebron, MD:	May 22
Dates of Termination:	Queenstown, MD:	Nov. 15
	Eden, MD:	Nov. 10
	Hebron, MD:	Nov. 12

As requested in the supplemental conditions included with the approval of permit # 97-098-02N, I am submitting a summary of the data collected from the field trial involving our [illegible] tolerant soybeans supervised by Mr. Billy Rhodes of Asgrow Seed Company. The purpose of this trial was to increase seed, advance generations, and evaluate agronomic characteristics.

Means of Containment and Plant Disposition: Machine planters were cleaned out after planting. A 20 foot buffer of soybeans was used to isolate the transgenic material at all locations. These plants were destroyed at the end of the trial and directly tilled into the soil. All seed harvested will be stored under approved conditions or shipped under permit to other Asgrow stations. After harvest, seed not used for further testing will be returned to the field, and incorporated by plowing or disking to eliminate volunteers. All residue was either left at the plot or returned to the plot, and incorporated by plowing or disking. The fields will be [illegible] soybeans next year and continuously monitored for volunteer plants.

Disease and Insect Susceptibility: No changes in morphology, [illegible] weediness was noticed in the transgenic soybeans.

Results: Soybean lines were advanced a generation and multiplied to provide seed for future Asgrow breeding and testing. Selections were made of the lines having the best phenotype.

Variety	Yield (bu/a)	Maturity	Height (in.)	Lodging
A5547	51.9	Oct. 23.5	38.5	2.9
L5143-127	52.6	Oct. 22.1	37.0	3.1
Std. Error	1.131	0.156	0.402	0.085

Asgrow Seed Company

Final Report to the USDA -- December 15, 1997

USDA Permit Number:	97-098-05N
Asgrow Permit Number:	AS041197
Applicant/Responsible Person:	Mr. Donald Steffen, Asgrow Seed Company
Locations:	The following locations were listed on the Field Release Notification, but were never planted (released): Marion, AR; Kennet, MO; Ripley, TN; Union City, TN; Brinkley, AR; Shoffner, AR; Cleveland, MS; Somerset, LA; and, Matthews, MO.
Responsible Researchers:	Mr. Larry Ganann, Mr. Will Winslow
Experiment Size:	No field releases (plantings) were performed.
Dates of Release:	No field releases (plantings) were performed.
Dates of Termination:	No field releases (plantings) were performed.

As requested in the supplemental conditions included with the approval of permit # 97-098-05N, I am submitting a summary of the data collected from the field trial involving our Ignite tolerant soybeans supervised by Mr. Larry Ganann and Mr. Will Winslow , both of Asgrow Seed Company. The purposes of the trial was to increase seed, advance generations, evaluate agronomic characteristics, assess weed control, and do further hybridization.

Means of Containment and Plant Disposition: No field releases (plantings) were performed.

Disease and Insect Susceptibility: No field releases (plantings) were performed

Results: No field releases (plantings) were performed

Asgrow Seed Company

Final Report to the USDA -- December 15. 1997

USDA Permit Number:	97-120-01N	
Asgrow Permit Number:	AS042897	
Applicant/Responsible Person:	Mr. Donald Steffen, Asgrow Seed Company	
Locations:	New Madrid, MO	
Responsible Researchers:	Mr. Tom Essary	
Experiment Size:	New Madrid, MO:	6.0 acres
	New Madrid, MO:	9.8 acres
	New Madrid, MO:	217.0 acres
Dates of Release:	New Madrid, MO:	May 20
	New Madrid, MO:	July 7
	New Madrid, MO:	June 21
Dates of Termination:	New Madrid, MO:	Oct. 17-18
	New Madrid, MO:	Nov. 8
	New Madrid, MO:	Oct. 24

As requested in the supplemental conditions included with the approval of permit # 97-120-01N, I am submitting a summary of the data collected from the field trial involving our lignite tolerant soybeans supervised by Mr. Tom Essary of Asgrow Seed Company. The purposes of the trial was to increase seed, advance generations, and evaluate agronomic characteristics.

Means of Containment and Plant Disposition: Machine planters were cleaned out after planting. A 20 foot buffer of soybeans was used to isolate the transgenic material at all locations. These plants were destroyed at the end of the trial and directly tilled into the soil. All seed harvested will be stored under approved conditions or shipped under permit to approved facilities stations. After harvest, seed not used for further testing will be returned to the [illegible] and incorporated by plowing or disking to eliminate volunteers. All residue was either left at the plot or returned to the plot and incorporated by plowing or disking. The fields will be planted to soybeans next year and continuously monitored for volunteer plants.

Disease and Insect Susceptibility: No [illegible] weediness was noticed in the transgenic [illegible].

Results: Soybean lines were advanced a generation and multiplied to provide seed for future Asgrow testing. No selections were made. Yield harvest data was collected at these sites in total bushels. These were large plots and no statistical data was calculated from these sites.

Location	Acreage	Bushels
New Madrid, MO:	6.0 acres	292.7 bu
New Madrid, MO:	9.8 acres	378.6 bu
New Madrid, MO:	217.0 acres	5026.0 bu

AgrEvo

98-014-01p

March 2, 1998

Dr. Shantu Shantharam
USDA, APHIS, PPQ, SS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

VIA FAX/CONFIRMATION COPY BY MAIL

Re: **Request for Extension of a Determination of Nonregulated Status for Glufosinate Resistant Soybean Transformation Events (96-068-1p)**

Dear Dr. Shantharam:

In accordance with our discussion on 26 February 1998 I am providing a description of the regulatory sequences found in transformation vector pB2/35SAcK and a comparison of the molecular data from GRS events. If these meet with your approval, then these pages will become part of the Appendix to the Extension. I have also provided a new Table of Contents showing these new portions to the Appendix, and a new Appendix page. These will replace the pages in the present submission (pages 5 and 28).

Please let me know at your earliest convenience whether these pages satisfy your request. AgrEvo USA will then forward two hard copies of each page for placement in the Extension document.

Please contact me at (302) 892-3155 if you have any questions or comments. Please leave a message as I will be out of the office this week.

Best Regards,

Sally Van Wert

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

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

3/11/98
original

Open Reading Frames and Associated Regulatory Regions in pB2/35SAcK

Vector pB2/35SAcK contains two open reading frames, *bla* and *pat*. Only the *pat* reading frame is functional and intact in the event A5547-127. The event is considered a regulated article because it contains 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 *bla* gene is also addressed. Refer to "Genetic Elements" table for a description of all other introduced genetic sequences in pB2/35SAcK.

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. *pat* 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 *pat* genes encode the enzyme phosphinothricin acetyltransferase (PAT), which imparts resistance to the phytotoxic activity of GA.

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-phosphinothricin moiety , is a potent inhibitor of GS (Bayer at el. 1972). L-phosphinothricin 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-phosphinothricin 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-phosphinothricin 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-phosphinothricin, the bacteria produce an inactivating enzyme, PAT. PAT catalyzes the conversion of L-phosphinothricin to N-acetyl-L-phosphinothricin in the presence of acetyl CoA as a co-substrate. N-acetyl-L-phosphinothricin 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).

3. *bla* The ß-lactamase 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.

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 plasmid 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)
bla	3876-3016	0.86	Ampicillin resistance gene from *E. coli* expresses a ß-lactamase only in bacteria (Sutcliffe, 1978).

Literature Cited

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Comparison of Molecular Data [1] for GRS Events: A2704-12, A2704-21, A5547-35 and A5547-127

Event	*pat* probe - # bands detected **HindIII digest**	*bla* probe - # bands detected **DraI digest**
A2704-12	4 [2]	2 [1, 4]
A2704-21	5 [2]	3 [1, 4]
A5547-35	1 [2]	0 [1, 4]
A5547-127	1 [3], 1 intact	2 [3, 4], 1- 3', 1-5'

(1) Data shown for shared GRS DNA event digests.
(2) From Southerns in petition # 96-068-01p.
(3) From Southern data presented in this extension.
(4) No intact *ampR* genes detected.

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VIII. Appendix -

USDA Field Trial Termination Reports
Open Reading Frames and Associated Regulatory Regions in pB2/35SAcK
Comparison of Molecular Data for GRS Events

Open Reading Frames and Associated Regulatory Regions in pB2/35SAcK

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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. *pat* 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 *pat* genes encode the enzyme phosphinothricin acetyltransferase (PAT), which imparts resistance to the phytotoxic activity of GA.

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-phosphinothricin moiety , is a potent inhibitor of GS (Bayer at el. 1972). L-phosphinothricin 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-phosphinothricin 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-phosphinothricin 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-phosphinothricin, the bacteria produce an inactivating enzyme, PAT. PAT catalyzes the conversion of L-phosphinothricin to N-acetyl-L-phosphinothricin in the presence of acetyl CoA as a co-substrate. N-acetyl-L-phosphinothricin 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).

3. *bla* The ß-lactamase 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.

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 plasmid 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)
bla	3876-3016	0.86	Ampicillin resistance gene from *E. coli* expresses a ß-lactamase only in bacteria (Sutcliffe, 1978).

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Comparison of Molecular Data [1] for GRS Events: A2704-12, A2704-21, A5547-35 and A5547-127

Event	*pat* probe - # bands detected **HindIII digest**	*bla* probe - # bands detected **DraI digest**
A2704-12	4 [2]	2 [1, 4]
A2704-21	5 [2]	3 [1, 4]
A5547-35	1 [2]	0 [1, 4]
A5547-127	1 [3], 1 intact	2 [3, 4], 1- 3', 1-5'

(1) Data shown for shared GRS DNA event digests.
(2) From Southerns in petition # 96-068-01p.
(3) From Southern data presented in this extension.
(4) No intact *ampR* genes detected.

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