This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 96–006–1]

Monsanto Co.; Addition of Two Genetically Engineered Insect Resistant Corn Lines to Determination of Nonregulated Status

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: The Animal and Plant Health Inspection Service is announcing that it has added two additional genetically engineered insect resistant corn lines to its August 22, 1995, determination that the Monsanto Company’s corn line MON 80100 need no longer be regulated. The effect of this action is that two additional insect resistant corn lines designated as MON 809 and MON 810, which have been modified by the incorporation of genetic material described by the Monsanto Company, will no longer be subject to regulation under 7 CFR part 340.

FOR FURTHER INFORMATION CONTACT: Dr. Ved Malik, Biotechnologist, Animal and Plant Health Inspection Service, Biotechnology, Biologics, and Environmental Protection, Biotechnology Permits, 4700 River Road Unit 147, Riverdale, MD 20737-1237; (301) 734-7612.

SUPPLEMENTARY INFORMATION: On September 5, 1995, the Animal and Plant Health Inspection Service (APHIS) published a notice in the Federal Register (60 FR 46107–46108. Docket No. 95–041–2) announcing the issuance of a determination effective August 22, 1995, that an insect resistant corn line developed by the Monsanto Company (Monsanto) designated as corn line MON 8010, does not present a plant pest risk and is not a regulated article under the regulations contained in 7 CFR part 340. This action was in response to a petition submitted by Monsanto seeking a determination from APHIS that its corn line MON 8010 no longer be deemed a regulated article, based on an absence of plant pest risk. The effect of that action was that the subject corn line and its progeny would no longer be regulated under the regulations in 7 CFR part 340.

The two additional corn lines that are the subject of this notice, MON 809 and MON 810, were identified in Monsanto’s previously submitted petition (APHIS Petition No. 95–093–01p) for corn line MON 80100. On January 17, 1996, APHIS received additional information and field test data in a petition (APHIS Petition No. 96–017–01p) in support of nonregulated status under 7 CFR part 340 for corn lines MON 809 and MON 810. As described by Monsanto, corn lines MON 809 and MON 810 express a CryIA(b) protein derived from the common soil bacterium Bacillus thuringiensis subspp. kurstaki which confers resistance to European corn borer. The subject corn lines were generated through use of the particle acceleration transformation system to insert plasmid vectors PV-ZMBK07 and PV-ZMGT10, the same vectors used to transform corn line MON 80100 for which the August 22, 1995, determination of nonregulated status was issued by APHIS.

Corn lines MON 809 and MON 810 have been evaluated in field tests conducted in 1993 and 1994 under APHIS permits and notifications. Reports from field trials and other data indicate that the subject corn lines grow normally, exhibit the expected morphological, reproductive, and physiological properties, and do not have unexpected pest or disease susceptibility or symptoms. Therefore, the APHIS determination of nonregulated status of August 22, 1995, applies as well to Monsanto’s two new transformed corn lines, MON 809 and MON 810.

Done in Washington, DC, this 11th day of March 1996.

Terry L. Medley,
acting Administrator, Animal and Plant Health Inspection Service.
Dr. Vedpal Malik  
Biotechnology Permit Unit  
Biotechnology, Biologics and Environmental Protection  
USDA-APHIS  
4700 River Road, Unit 147  
Riverdale, MD 20737-1237

Dear Dr. Malik:

Please find enclosed the Southern data in support of the molecular analysis for YieldGard corn lines MON 809 and 810. This additional information is submitted in support of Monsanto's USDA petition for non-regulated status for additional corn lines MON 809 and MON 810 received by the USDA on January 17, 1996 and identified as petition 96-017-01p. These lines were previously identified in USDA petition 95-093-01p which provided non-regulated status for line MON 80100 dated August 22, 1995 (FR 60:171; pp. 46107-46108). Approval of lines MON 809 and 810 has been requested in connection with this previous approval.

Sincerely,

[Signature]

Kent A. Croon, Ph.D.  
Regulatory Affairs Manager
Molecular Analysis of YieldGard™ Corn Line MON 809

Janice Kania, Pamela Keck and Patricia Sanders

I. SUMMARY

This report describes the molecular analysis of the integrated DNA (I-DNA) present in YieldGard™ corn line MON 809. Specifically, the insert number (number of integration sites within the corn genome) and the number and integrity of each inserted gene were determined. The corn line MON 809 was produced by particle acceleration technology with two plasmids PV-ZMBK07 [cryIA(b) gene] and PV-ZMGT10 [CP4 EPSPS and gox genes]. Corn line MON 809 contains one I-DNA of approximately 23 Kb which includes either complete or partial genes of cryIA(b), CP4 EPSPS and gox. The I-DNA contains two cryIA(b) genes, one which is the correct size, (3.46 Kb), and one which is smaller (less than 1.0 Kb). There are two CP4 EPSPS genes, both of expected size (1.3 Kb). The single gox gene present in corn line MON 809 is not intact. The nptII and ori-pUC probings showed that the backbone was present in the YieldGard™ corn line MON 809, but was not the predicted size. Based on these analyses, we conclude that corn line MON 809 contains a single I-DNA with an intact cryIA(b) gene and two CP4 EPSPS genes that are responsible for producing the correct size CryIA(b) and CP4 EPSPS proteins.

<table>
<thead>
<tr>
<th>Summary of Corn Line MON 809 Molecular Analysis</th>
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<tbody>
<tr>
<td>Genetic Element</td>
</tr>
<tr>
<td>cryIA(b) gene</td>
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<tr>
<td>CP4 EPSPS gene</td>
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<tr>
<td>gox gene</td>
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<tr>
<td>nptII/ori-pUC</td>
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</table>

II. RESULTS AND DISCUSSION

A. Southern blot results

Two plasmid vectors were utilized during the particle acceleration process to produce the corn line MON 809. Plasmid PV-ZMBK07 contained the cryIA(b) gene and plasmid PV-ZMGT10 contained the CP4 EPSPS and gox genes. The maps of the two plasmid vectors are presented in Figure 1, along with the locations of the restriction sites utilized for Southern analyses.

The DNAs from MON 818 and MON 809 plants were digested with a variety of restriction enzymes and subjected to Southern blot hybridization analyses to characterize the DNA that was stably transferred during the particle acceleration into the corn genome. Specifically, the insert number (number of integration sites within the corn genome), and the copy number and integrity of each inserted gene was examined.
B. Insert Number

NdeI digestion results. The purpose of the NdeI digests was to determine the number of plasmid DNA inserts in the corn line MON 809. The plasmids PV-ZMBK07 and PV-ZMGT10 do not contain a restriction site for NdeI. Thus this enzyme effectively cuts outside any inserted DNA, releasing a fragment containing the inserted DNA. MON 818 control DNA and MON 809 DNA were digested with NdeI and probed with the cryIA(b) gene, the CP4 EPSPS gene and the gox gene. The results are shown in Figure 2. Lanes 1, 3 and 5 contain MON 818 control DNA. No bands were observed, as expected, when probed with the cryIA(b), CP4 EPSPS or gox genes. MON 809 DNA produced one band, approximately 23 Kb in size, when probed with: the cryIA(b) gene (lane 2), the CP4 EPSPS gene (lane 4) and the gox gene (lane 6). The band produced in the gox gene probing is very faint and only observed with long exposure times, suggesting that only a portion of the gox gene is present in the inserted DNA of YieldGard™ corn line MON 809.

C. Insert Composition

1. cryIA(b) gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the cryIA(b) gene in MON 809 and the Southern blot probed with the cryIA(b) gene. The results are shown in Figure 3, lanes 1-3. The MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 (lane 2). The MON 818 DNA (lane 1) produces two faint bands, approximately 2.5 Kb and 1.9 Kb in size. These bands are considered to be background bands since they are observed in all three lanes and are not discussed further. The MON 818 DNA mixed with plasmids (lane 2) produced one new 3.46 Kb fragment which corresponds to the expected size of the intact cryIA(b) gene (refer to the PV-ZMBK07 plasmid map in Fig. 1). The MON 809 DNA (lane 3) contains two bands, 3.46 Kb and 1.0 Kb. The 3.46 Kb band is the expected size band for an intact cryIA(b) gene, and the 1.0 Kb band represents a partial cryIA(b) gene. The NcoI/EcoRI digests, probed with the cryIA(b) gene, identified one intact and one partial cryIA(b) gene.

2. CP4 EPSPS gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the CP4 EPSPS gene in MON 809 DNA and the Southern blot probed with the CP4 EPSPS gene. The results are shown in Figure 4, lanes 1-3. The MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 also digested with NcoI/EcoRI (lane 2). The MON 818 DNA (lane 1) showed two bands, approximately 1.37 Kb and 0.80 Kb in size. These two bands, present in all three lanes, are background bands and are therefore not considered further. The MON 818 DNA mixed with the plasmids (lane 2) produced an additional band, 1.06 Kb, which is the expected size of the CP4 EPSPS gene, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). The MON 809 DNA (lane 3) also contains a band of 1.06 Kb, the expected size band for the CP4 EPSPS gene. This band contains two expected size CP4 EPSPS genes that are present in
corn line MON 809 (data not shown). The Ncol/EcoRI digests, probed with the CP4 EPSPS gene, identified only the expected size CP4 EPSPS gene.

3. gox gene integrity. MON 818 and MON 809 DNAs were digested with Ncol/EcoRI to release the gox gene in MON 809 DNA and the Southern blot probed with the gox gene. The results are shown in Fig. 5, lanes 1-3. MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 also digested with Ncol/EcoRI (lane 2). The MON 818 DNA (lane 1) does not show any bands, as expected for the control DNA. The MON 818 DNA mixed with the plasmids (lane 2) produces a 1.3 Kb band, which corresponds to the expected size of the intact gox gene, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). The MON 809 DNA (lane 3) contains one band of 3.5 Kb. The 3.5 Kb band is faint and was observed only with long exposure times. The faintness of the gox band suggests that only a part of the gox gene is present. The larger than predicted Ncol/EcoRI fragment size (3.5 Kb rather than 1.3 Kb) indicates a DNA rearrangement has occurred within the gox gene. Corn line MON 809 appears to contain a partial gox gene.

4. Backbone integrity. MON 818 and MON 809 DNAs were digested with NotI to release the intact nptII/ori-pUC backbone in MON 809 DNA and the Southern blot probed with the nptII gene. The results are shown in Figure 6 (lanes 1 and 2). The digested MON 818 DNA was mixed with 15 pg of PV-ZMBK07 and PV-ZMGT10 also digested with NotI. The MON 818 DNA and plasmid mixture contains two bands of 5.9 Kb and 2.6 Kb (lane 1). The 5.9 Kb band corresponds to the expected size band of the intact backbone from PV-ZMGT10, the 2.6 Kb band corresponds to the expected size band of the intact backbone from PV-ZMBK07 (refer to Fig. 1). The MON 809 DNA contains a 4.2 Kb band (lane 2) which hybridized to the nptII probe.

The Southern blot was stripped and reprobed with the ori-pUC genetic region. The MON 818 DNA and plasmid mixture (lane 3) contains three bands of 5.9 Kb, 4.2Kb and 2.6 Kb. The 5.9 Kb band corresponds to the expected size band of the intact backbone from PV-ZMGT10, the 2.6 Kb band corresponds to the expected size band of the intact backbone from PV-ZMBK07 (refer to Fig. 1). The 4.2 Kb band is a background band. The MON 809 DNA contains one band, 4.2 Kb in size (lane 4) which corresponds to the band which hybridized to the nptII gene in lane 2. The 4.2 Kb background band (lane 3) co-migrates with the one band which hybridized to the nptII and ori-pUC probes (lane 4). The 4.2 Kb band hybridized to the nptII and ori-pUC probes, indicating that the backbone is present but is not the predicted size.
III. CONCLUSIONS

The corn line MON 809 was produced by particle acceleration technology with the two plasmids PV-ZMBK07 and PV-ZMGT10 that contained the crylA(b), CP4 EPSPS, gax and nptII genes. The I-DNA (23 Kb) contains two crylA(b) genes, one which is the correct size, (3.46 Kb), and one which is smaller (less than 1.0 Kb). There are two CP4 EPSPS genes, both of expected size (1.3 Kb). The gax gene present in corn line MON 809 is not intact. The nptII and ori-pUC probings showed that the backbone was present in the corn line MON 809, but was not the predicted size.

Based on these analyses, we conclude that corn line MON 809 contains a single I-DNA with an intact crylA(b) gene and two CP4 EPSPS genes that are responsible for producing the correct size CrylA(b) and CP4 EPSPS proteins.
Figure 1. Plasmid maps of PV-ZMBK07 and PV-ZMGT10. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.
Figure 2. Southern blot analysis of corn line MON 809 DNA: insert number analysis

Figure 2. Southern blot analysis of corn line MON 809 DNA. Lanes 1, 3 and 5 contain 12.5 µg of corn line MON 818 DNA digested with NdeI. Lanes 2, 4 and 6 contain 12.5 µg of corn line MON 809 DNA digested with NdeI. Lanes 1 and 2 were hybridized with the cryIA(b) gene. Lanes 3 and 4 were hybridized with the CP4 EPSPS gene. Lanes 5 and 6 were hybridized with the gox gene.

Symbol denotes sizes obtained from MW markers.
Figure 3. Southern blot analysis of corn line MON 809 DNA: cryIA(b) gene analysis

Figure 3. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the cryIA(b) gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

Symbol denotes sizes obtained from MW markers on ethidium stained gel.
Symbol denotes sizes obtained from plasmid digests.
Symbol denotes background bands (≈2.5 and 1.9 Kb).
Symbol denotes a band size approximated from MW marker and plasmid digests.
Symbol denotes an area of non-specific hybridization. This is supported by the observation that the signal is between two lanes.
Figure 4. Southern blot analysis of corn line MON 809 DNA: CP4 EPSPS gene analysis

MW markers

23.1 Kb —
9.42 Kb —
6.56 Kb —
4.36 Kb —

2.32 Kb —
2.03 Kb —

1.35 Kb —
1.08 Kb —

0.87 Kb —

NcoI/EcoRI
CP4 EPSPS

Figure 4. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the CP4 EPSPS gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMG10; lane 3, MON 809 DNA.

Symbol denotes sizes obtained from MW markers on ethidium stained gel.
Symbol denotes sizes obtained from plasmid digests.
Symbol denotes background bands (~1.37 and 0.80 Kb).
Symbol denotes a band size approximated from MW marker and plasmid digests.
Figure 5. Southern blot analysis of corn line MON 809 DNA: gox gene analysis

Figure 5. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the gox gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

Symbol denotes sizes obtained from MW markers on ethidium stained gel.
Symbol denotes sizes obtained from plasmid digests.
Symbol denotes a band size approximated from MW marker and plasmid digests.
Figure 6. Southern blot analysis of corn line MON 809 DNA: nptII and ori-pUC analysis.

Figure 6. Southern blot analysis of corn line MON 809 DNA. Lanes 1-4 contain the following DNAs digested with NotI: lanes 1 and 3, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lanes 2 and 4, MON 809 DNA. Lanes 1 and 2 were hybridized with the nptII region. Lanes 3 and 4 were hybridized with the ori-pUC region.

Symbol denotes sizes obtained from MW markers on ethidium stained gel.
Symbol denotes sizes obtained from plasmid digests.
Symbol denotes background bands.
Symbol denotes a band size approximated from MW marker and plasmid digests.
Molecular Analysis of Insect Protected Maize Line MON 810

Janice Kania, Pamela Keck, Elaine Levine and Patricia Sanders

I. SUMMARY

This report describes the molecular analysis of the integrated DNA in Insect Protected maize line MON 810. Specifically, the insert number (number of integration sites within the maize genome) and the number and integrity of the inserted genes were determined. Maize line MON 810 was produced by particle acceleration technology using a DNA solution containing two plasmids, PV-ZMBK07 and PV-ZMGT10. The maize transformation vectors used to produce maize line MON 810 contain genes encoding 1) cryIA(b) gene; 2) CP4 5-enolpyruvyl-shikimate-3-phosphate synthase (CP4 EPSPS); 3) glyphosate oxidoreductase (gox); and 4) the nptII gene, under the control of a bacterial-specific promoter. Molecular analysis of maize line MON 810 established that the line only contains the cryIA(b) gene from plasmid PV-ZMBK07. The line does not contain the CP4 EPSPS, gox, or nptII genes. There is no evidence that any of the DNA contained in plasmid PV-ZMGT10 was inserted. Maize line MON 810 contains one integrated DNA, contained on a 5.5 Kb Ndel fragment, which contains the E35S promoter, maize hsp70 intron and the cryIA(b) gene.

<table>
<thead>
<tr>
<th>Genetic Element</th>
<th>Maize Line MON 810</th>
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<tbody>
<tr>
<td>cryIA(b) gene</td>
<td>present</td>
</tr>
<tr>
<td>CP4 EPSPS gene</td>
<td>not present</td>
</tr>
<tr>
<td>gox gene</td>
<td>not present</td>
</tr>
<tr>
<td>nptII/ori-pUC</td>
<td>not present</td>
</tr>
</tbody>
</table>

II. RESULTS AND DISCUSSION

A. Southern blot results

Plasmid PV-ZMBK07 contained the cryIA(b) gene and plasmid PV-ZMGT10 contained the CP4 EPSPS and gox genes. The maps of the two plasmid vectors, along with the locations of the restriction sites utilized for Southern analyses, are presented in Figure 1.

The DNAs from MON 818 and MON 810 plants were digested with a variety of restriction enzymes and subjected to Southern blot hybridization analyses to characterize the DNA that was transferred during the particle acceleration into the maize genome. Specifically, the insert number (number of integration sites within the maize genome), and the copy number and integrity of each gene was examined.
B. Insert Number

NdeI digestion results. The purpose of the NdeI digests was to determine the number of plasmid DNA inserts in the maize line MON 810. The plasmids PV-ZMBK07 and PV-ZMG10 do not contain a restriction site for NdeI. Thus this enzyme effectively cleaves outside any inserted DNA, releasing a fragment containing the inserted DNA and adjacent genomic DNA. MON 818 control DNA and MON 810 DNA were digested with NdeI and probed with plasmid PV-ZMBK07 DNA. The results are shown in Figure 2. MON 818 DNA (lane 1), produced one very light, diffused band of approximately 21.0 Kb which is a background band since it is present in both the control MON 818 DNA and the MON 810 DNA. MON 810 DNA produced one band, approximately 5.5 Kb in size (lane 2). This result established that insect protected maize line MON 810 contains one fragment of integrated DNA. The size of the inserted DNA plus adjacent genomic DNA up to the NdeI restriction sites is approximately 5.5 Kb.

C. Insert Composition

1. cryIA(b) gene integrity. MON 818 and MON 810 DNAs were digested with NcoI/EcoRI to release the cryIA(b) gene and the Southern blot probed with the cryIA(b) gene. The results are shown in Figure 3, lanes 1-3. The positive hybridization control (lane 1) produced one 3.46 Kb fragment which corresponds to the expected size of the cryIA(b) gene (refer to the plasmid maps in Fig. 1). Due to the plasmid DNA not being mixed with genomic control DNA the band appears larger than its true molecular weight. The MON 818 DNA (lane 2) does not produce any bands, as expected for the control line. The MON 810 DNA (lane 3) contains one band, approximately 3.1 Kb.

2. CP4 EPSPS gene integrity. Plasmid DNAs (PV-ZMBK07 and PV-ZMG10) and insect protected maize line MON 810 DNA were digested with NcoI/BamHI to release the CP4 EPSPS gene and the Southern blot probed with the CP4 EPSPS gene. The results are shown in Figure 4, lanes 1 and 2. Approximately 50 pg of a mixture of PV-ZMBK07 and PV-ZMG10 DNA (lane 1) produced one band, approximately 3.1 Kb in size, which corresponds to the expected size CP4 EPSPS fragment, as predicted from the plasmid map (PV-ZMG10 in Fig. 1). MON 810 DNA (lane 2) shows no hybridizing fragments to the CP4 EPSPS probe, establishing that insect protected maize line MON 810 does not contain the CP4 EPSPS gene.

3. gox gene integrity. Plasmid DNAs (PV-ZMBK07 and PV-ZMG10) and insect protected maize line MON 810 DNA were digested with NcoI/BamHI to release the gox gene and the Southern blot probed with the gox gene. The results are shown in Figure 4, lanes 3 and 4. Approximately 50 pg of a mixture of PV-ZMBK07 and PV-ZMG10 DNA (lane 3) produced one band, a NcoI/NcoI fragment, approximately 3.1 Kb, which corresponds to the expected size gox fragment, as predicted from the plasmid map (PV-ZMG10 in Fig. 1). MON
810 DNA (lane 4) shows no hybridizing fragments to the gox probe, establishing that insect protected maize line MON 810 does not contain the gox gene.

4. Backbone integrity. Plasmid PV-ZMBK07, control line MON 818 and insect protected maize line MON 810 DNAs were digested with NcoI/EcoRI to release the nptII/ori-pUC backbone and the Southern blot probed with the nptII gene. The results are shown in Figure 5 (lanes 1-3). Approximately 50 pg of PV-ZMBK07 DNA produced two bands of 2.5 Kb and 1.8 Kb (lane 1). The 2.5 Kb and 1.8 bands correspond to the expected size fragments of the backbone from vector PV-ZMBK07 (refer to Fig. 1). The MON 818 DNA alone (lane 2) does not produce any bands, as expected from a non-modified control line. MON 810 DNA (lane 3) shows no bands, establishing that the backbone sequences were not integrated in insected protected maize line MON 810.

The Southern blot was stripped and reprobed with the ori-pUC genetic region. The PV-ZMBK07 and PV-ZMGT10 DNAs (lane 4) contains one band of 1.8 Kb. The 1.8 Kb band corresponds to the expected size fragment of the backbone from PV-ZMBK07 (refer to Fig. 1). The MON 818 DNA alone (lane 5) does not produce any bands, as expected for the unmodified control line. MON 810 DNA (lane 6) shows no bands, establishing that the backbone sequences were not integrated in insected protected maize line MON 810. The lack of observed bands with both ori-pUC and nptII probes, established that insect protected maize line MON 810 does not contain any backbone sequences.

V. CONCLUSIONS

The insect protected maize line MON 810 was produced by particle acceleration technology with a DNA solution that contained the cryIA(b), CP4 EPSPS, gox and nptII genes. Maize line MON 810 contains one integrated DNA contained on a 5.5 Kb NdeI fragment, which contains the E35S promoter, maize hsp70 intron and the cryIA(b) gene. Insect protected maize line MON 810 does not contain a CP4 EPSPS gene, a gox gene or nptII/ori-pUC sequences. The continued efficacy of maize line MON 810 confirms that an insecticidally active CryIA(b) protein is produced which provides season long control of European Corn Borer.
Figure 1. Plasmid maps of PV-ZMBK07 and PV-ZMGT10. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.
Figure 2. Southern blot analysis of maize line MON 810 DNA: insert number analysis

23.0 Kb →
9.42 Kb →
6.56 Kb →
4.36 Kb →
2.32 Kb →
2.03 Kb →
1.35 Kb →
1.08 Kb →
0.87 Kb →

NdeI
PV-ZMBK07

Figure 2. Southern blot analysis of maize line MON 810 DNA. Lanes 1 and 2 contain the following DNAs digested with NdeI and probed with PV-ZMBK07: lane 1, MON 818 DNA; lane 2, MON 810 DNA.

Symbol denotes sizes obtained from MW markers.
Symbol denotes a band size approximated from MW marker and plasmid digests.
Symbol denotes background bands.
Figure 3. Southern blot analysis of maize line MON 810 DNA: cryIA(b) gene analysis

Figure 3. Southern blot analysis of maize line MON 810 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the cryIA(b) gene: lane 1, 50 pg of plasmid PV-ZMBK07; lane 2, MON 818 DNA, lane 3, MON 810 DNA.

Symbol denotes sizes obtained from MW markers on ethidium stained gel.
Symbol denotes sizes obtained from plasmid digests.
Symbol denotes a band size approximated from MW marker and plasmid digests.
Symbol denotes an area of hybridization in an adjacent lane which only appears to be in lane 1, due to the contents of the lanes migrating at an angle in this portion of the gel.
Figure 4. Southern blot analysis of maize line MON 810 DNA: CP4 EPSPS and gox gene analysis

Figure 4. Southern blot analysis of maize line MON 810 DNA. Lanes 1-4 contain the following DNAs digested with NcoI/BamHI: lanes 1 and 3, ~50pg of plasmids PV-ZMGT10 and PV-ZMBK07; lanes 2 and 4, MON 810 DNA. Lanes 1 and 2 were hybridized with the CP4 EPSPS gene. Lanes 3 and 4 were hybridized with the gox gene.

Symbol denotes sizes obtained from MW markers on ethidium stained gel.
Symbol denotes sizes obtained from plasmid digests.
Figure 5. Southern blot analysis of maize line MON 810 DNA: nptII and ori-pUC analysis

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Figure 5. Southern blot analysis of maize line MON 810 DNA. Lanes 1-6 contain the following DNAs digested with NcoI/EcoRI: lanes 1 and 4, ~50pg of plasmid PV-ZMBK07; lanes 2 and 5, MON 818 DNA; lanes 3 and 6, MON 810 DNA. Lanes 1-3 were hybridized with the nptII region. Lanes 4-6 were hybridized with the ori-pUC region.

 Symbol denotes sizes obtained from MW markers on ethidium stained gel.
 Symbol denotes sizes obtained from plasmid digests.
Subject: Petition for Determination of Non-Regulated Status: Additional YieldGard™ Corn (Zea mays L.) Lines with the cryIA(b) Gene from Bacillus thuringiensis subsp. kurstaki.
Monsanto #: 95-274U

Dear Mr. Lidsky:

The Agricultural Group of Monsanto Company is submitting a Petition for Determination of Non-Regulated Status to the Animal and Plant Health Inspection Service (APHIS) regarding additional corn lines which express a CryIA(b) protein derived from the common soil bacterium Bacillus thuringiensis subsp. kurstaki (B.t.k.). Field experiments were conducted in 1993 and 1994 in the U.S. corn growing region under United States Department of Agriculture (USDA) permits or notifications as well as an Experimental Use Permit (524-EUP-82) obtained from the EPA in 1994 and renewed in 1995. Results from these field experiments have demonstrated that YieldGard corn lines MON 809 and 810 are protected season long from the leaf and stalk feeding damage caused by European corn borer (Ostrinia nubilalis).

This petition requests a determination from APHIS that YieldGard™ corn lines MON 809 and 810, any progenies derived from crosses between MON 809 and 810 and traditional corn varieties, and any progeny derived from crosses of MON 809 and 810 with transgenic corn varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under regulations in 7 CFR part 340. These two additional corn lines were originally identified in USDA Petition 95-093-01p for YieldGard corn line MON 80100 submitted to the agency on March 30, 1995 and approved August 22, 1995 (FR 60:171; pp. 46107-46108).
We appreciate your attention to this matter. Should you have any questions, please feel free to contact either Dr. Dickerson at 202-383-2857 or myself (314-537-7488).

Sincerely,

[Signature]

Kent A. Croon, Ph.D.
Regulatory Affairs Manager

cc: Dr. C.T. Dickerson - Monsanto
Petition for Determination of Nonregulated Status:

Additional YieldGard™ Corn (Zea mays L.) Lines with the cryIA(b) Gene from Bacillus thuringiensis subsp. kurstaki

The undersigned submits this petition of 7 CFR 340.6 to request that the Director, BBEP, make a determination that additional lines of YieldGard™ corn should not be a regulated article under 7 CFR part 340.

Submitted by:

[Signature]
Kent A. Croon, Regulatory Affairs Manager
Ceregen, a Unit of Monsanto Company, BB3A
700 Chesterfield Parkway North
Chesterfield, MO 63198
Tel: 314-537-7488
Fax: 314-537-7085

January 8, 1996
#95-274U

Prepared by:

K.A. Croon, P.R. Sanders, J. Kania, P. Keck, E. Levine,
and G.B. Parker
Additional YieldGard™ Corn (*Zea mays* L.) Lines with the cryIA(b) Gene from *Bacillus thuringiensis* subsp. *kurstaki*

Summary

Monsanto Company is submitting this Petition for Determination of Non-regulated Status to the Animal Plant Health Inspection Service (APHIS) regarding additional corn lines which express a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). This petition requests a determination from APHIS that YieldGard™ corn lines MON 809 and 810, any progenies derived from crosses between MON 809 and 810 and traditional corn varieties, and any progeny derived from crosses of MON 809 and 810 with transgenic corn varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under regulations in 7 CFR part 340. These two additional corn lines were originally identified in USDA Petition 95-093-01p for YieldGard corn line MON 80100 submitted to the agency on March 30, 1995 and approved August 22, 1995 (FR 60:171; pp. 46107-46108).

Field experiments were conducted in 1993 and 1994 in the U.S. corn growing region under United States Department of Agriculture (USDA) permits or notifications as well as an Experimental Use Permit (524-EUP-82) obtained from the EPA in 1994 and renewed in 1995. Results from these field experiments have demonstrated that YieldGard corn lines MON 809 and 810 are protected season long from the leaf and stalk feeding damage caused by European corn borer (*Ostrinia nubilalis*). Growers planting YieldGard corn will not require insecticide applications to control European corn borer (ECB). This reduction in insecticide use will enhance biological control and the implementation of other pest management strategies for other corn pests. In addition, these plants exhibit no pathogenic properties, are no more likely to become weeds than the non-modified parental corn lines, are unlikely to increase the weediness potential for any other cultivated plants or native species, and are equivalent morphologically, agronomically, and compositionally to the parental corn lines.

The use of YieldGard corn will have a more positive impact on the environment than the use of chemical insecticides to control ECB. The CryIA(b) protein is ecologically benign, i.e., it breaks down rapidly in the soil.

YieldGard™ is a registered trademark of Monsanto Company, St. Louis, MO.
and is safe to non-target organisms such as fish, birds, mammals, and beneficial insects. In addition, the risk of an uncontrolled introduction of this corn into the environment through hybridization or outcrossing to native species is virtually non-existent in the U.S.

In conclusion, the consistent control afforded by YieldGard corn lines MON 809 and 810 will enable growers to significantly reduce the amount of chemical insecticide now applied to their crop for control of ECB while maintaining yield potential. As a result, they will be able to utilize IPM practices that cannot presently be implemented because of the lack of options other than use of chemical insecticides to control this pest. An increase in the biological and cultural control of non-target corn pests and a more judicious use of chemical insecticides will result in a positive impact on the environment, which will ultimately be advantageous to the grower and the public as well.

Therefore, Monsanto Company requests a determination from APHIS that YieldGard corn lines MON 809 and 810 and any progenies derived from crosses between MON 809 and 810 and traditional corn varieties no longer be considered regulated articles under regulations in 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.

[Signature]

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<td>APHIS</td>
<td>Animal Plant Health Inspection Service</td>
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<tr>
<td>bp, Kb</td>
<td>Base pairs, kilobase pairs</td>
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<td>B.t.k.</td>
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<td>CaMV</td>
<td>Cauliflower mosaic virus</td>
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<tr>
<td>CFR</td>
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<td>CrylA(b)</td>
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<td>FFDCA</td>
<td>Federal Food Drug and Cosmetic Act</td>
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<td>FIFRA</td>
<td>Federal Insecticide Fungicide and Rodenticide Act</td>
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<td>GLP</td>
<td>Good Laboratory Practice</td>
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<td>gox</td>
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<td>Neomycin phosphotransferase II</td>
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<td>USDA</td>
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<td>µg, g</td>
<td>Microgram, gram</td>
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Part I. Rationale for Development of YieldGard™ Corn

A. Need and Benefits of Yieldgard Corn

Corn is the largest U.S. crop in terms of acreage, total production, and crop value (National Corn Growers Association, 1994). European corn borer (ECB) (*Ostrinia nubilalis*) is among the most important corn insect pests in the U.S. and worldwide (Dicke and Guthrie, 1988). This pest ranges from the Eastern seashore west to the Rocky Mountains and from southern Canada to Florida and the Gulf States. In the central corn belt, the pest typically completes two generations each year, but in warm years may complete a partial to full third generation (USDA, 1992). Physical damage results from ECB as a result of: (1) leaf feeding (from the first generation), (2) stalk tunneling (from the first and second generation), (3) leaf sheath and collar feeding (from the second and third generation) and (4) ear damage (from the second and third generation) (USDA, 1992). Researchers from across the pest’s geographic range have estimated a five to ten percent corn yield loss annually, attributable to ECB damage (USDA Petition 95-093-01p; Bode and Calvin, 1990; Guthrie *et al.*, 1975; Rice, 1994a-c). Yield losses are attributed to disruption of nutrient and water translocation to key tissues, secondary disease infections, stalk lodging, ear droppage and kernel damage.

Control of ECB using conventional insecticide applications is variable due to difficulties in the proper timing of the application and placement of the insecticide where ECB larvae are feeding. Small deviations from the optimal date for applying an insecticide can result in significantly less control. More than one insecticide application may be necessary. To time these insecticide applications properly, a field scouting program is required (USDA, 1992; USDA Petition 95-093-01p). Hybrids with resistance to the first generation (leaf-feeding resistance) of ECB, obtained through traditional breeding techniques, can reduce the amount of loss. However, to date, these hybrids do not have the yield potential of susceptible full-season hybrids (USDA, 1992).

Monsanto has developed genetically modified corn plants (YieldGard™) that control ECB. This YieldGard corn offers a new mechanism to produce and deliver a highly effective insecticide to target pests (e.g. production by cells of the crop plant rather than industrially and application by spray equipment). The technology couples the environmental advantages of host plant resistance with the efficacy of CryIA(b), an effective biological
insecticide. YieldGard corn expresses the CryIA(b) protein which is selective against certain lepidopteran insects that must feed upon the plants to be controlled. Therefore, this technology offers selective activity without disrupting pest suppression by natural enemies, such as parasites and predators.

The determination that YieldGard corn lines MON 809 and 810 and their progenies are no longer regulated articles and their subsequent commercialization will represent an efficacious and environmentally compatible addition to the existing options for corn insect pest management. The use of YieldGard corn will provide potential benefits to growers, the general public and the environment, including:

- A more reliable, economical, and less labor intensive means to control ECB.

- Insect control without harming non-target species, including humans.

- A means for growers to significantly reduce the amount of chemical insecticides now applied to the crop thereby achieving ECB control in a more environmentally compatible manner than is currently available.

- A reduction in the manufacturing, shipment, and storage of chemical insecticides used in corn.

- A reduction in the exposure to workers to the pesticide and pesticide spray solution.

- A reduction in the number of empty pesticide containers and amount of pesticide spray solution that must be disposed of according to applicable environmental regulations.

- An ideal fit with Integrated Pest Management (IPM) and sustainable agricultural systems.

- Both large and small growers will benefit from the planting of YieldGard corn as no additional labor, planning, or machinery is required.
B. Regulatory Approvals

Before commercializing YieldGard corn lines MON 809 and 810, Monsanto will seek the following regulatory approvals:

1. This determination from USDA/APHIS that YieldGard corn lines MON 809 and 810, and all progenies from crosses between YieldGard corn lines MON 809 and 810 and other corn varieties, are no longer a regulated article according to 7CFR §340.6.

2. Regulatory approval from the Environmental Protection Agency (EPA) of the CryIA(b) insecticidal protein as expressed in YieldGard corn under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). This petition has been submitted.

3. An exemption from the requirement of a tolerance for the CryIA(b) insecticidal protein, the CP4 EPSPS selectable marker enzyme, and the genetic material necessary for the production of these proteins in or on all agricultural commodities under sections 408 of the Federal Food Drug and Cosmetic Act (FFDCA) from the EPA.

In addition, we will complete our consultations which have been initiated with the FDA under their May 29, 1992 policy statement concerning foods derived from new plant varieties.

Monsanto will consult with the pesticide and, if applicable, biotechnology regulatory officials of the states in which the commercial product will be sold and obtain a state license, if such is required.
C. References


USDA Petition 95-093-01p. Insect Protected Corn (Zea mays L.) with the cryIA(b) Gene from Bacillus thuringiensis subsp. kurstaki. FR 60:171 pp. 46107-46108.
Part II. The Corn Family

A. Summary

Corn (*Zea mays* L.), or maize, is one of the few major crop species indigenous to the Western Hemisphere (Goodman, 1988). Corn is grown in nearly all areas of the world and ranks third behind rice (*Oryza sativa* L.) and wheat (*Triticum* sp.) in total production. Corn has been studied extensively, and it seems the probable domestication of corn was in southern Mexico more than 7,000 - 10,000 years ago (Gould, 1968; Galinat, 1988; Jungenheimer, 1976). The putative parents of corn have not been recovered, but it seems teosinte probably played an important role in the genetic background of corn (Mangelsdorf, 1974).

The transformation from a wild, weedy species to one dependent on humans for its survival probably evolved over a long period of time by the indigenous inhabitants of the Western Hemisphere. Corn, as we known it today, cannot survive in the wild, because the female inflorescence (the ear) restricts seed dispersal (Galinat, 1988; Goodman, 1988; Mangelsdorf, 1986; Wilkes, 1986). Although grown extensively throughout the world, corn is not considered a persistent weed nor one difficult to control.

A summary of the history, taxonomy, genetics, life cycle, and potential gene flow of corn is located in USDA petition 95-093-01p as prepared by Dr. Arnel R. Hallauer, Department of Agronomy, Iowa State University, Ames, Iowa.
B. References


Part III. Description of the Transformation System and Plasmids Utilized

A. Construction of the Plasmid Vectors, PV-ZMBK07 and PV-ZMGT10, Utilized for Transformation

YieldGard corn lines MON 809 and 810 were produced with a DNA solution containing two plasmid vectors, PV-ZMBK07 and PV-ZMGT10. Plasmid DNA was introduced into the plant tissue using the particle acceleration method as previously identified (USDA Petition 95-093-01p). The PV-ZMBK07 plasmid contains the cryIA(b) gene and PV-ZMGT10 contains the CP4 EPSPS and gox genes. Both plasmids contain the nptII gene under the control of a bacterial promoter and an origin of replication from a pUC plasmid, required for selection and replication in bacteria, respectively. The plasmid vector PV-ZMBK07 is shown in Figure III.1 and PV-ZMGT10 is shown in Figure III.2. A description of the DNA elements in PV-ZMBK07 and PV-ZMGT10 are provided in Tables III.1 and III.2, respectively.
Figure III.1 Plasmid map of PV-ZMBK07.
Figure III.2 Plasmid map of PV-ZMGT10.
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<tr>
<th>Genetic Element</th>
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<td>E35S</td>
<td>0.61</td>
<td>The cauliflower mosaic virus (CaMV) promoter (Odell et al., 1985) with the duplicated enhancer region (Kay et al., 1985).</td>
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<td>hsp70 intron</td>
<td>0.80</td>
<td>Intron from the maize hsp70 gene (heat-shock protein) present to increase the level of gene transcription (Rochester et al., 1986).</td>
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<td>cryIAb</td>
<td>3.46</td>
<td>The gene encodes the nature identical CryIAb protein product (Fischhoff et al., 1987).</td>
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<td>A 3' nontranslated region of the nopaline synthase gene which terminates transcription and directs polyadenylation (Fraley et al., 1983).</td>
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<td>lacZ</td>
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<td>ori-pUC</td>
<td>0.65</td>
<td>The origin of replication for the pUC plasmids that allows for plasmid replication in E. coli (Vieira and Messing, 1987).</td>
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<td>nptII</td>
<td>0.79</td>
<td>The gene for the enzyme neomycin phosphotransferase type II. This enzyme confers resistance to aminoglycoside antibiotics and thereby allows for selection of bacteria containing the plasmid (Beck et al., 1982).</td>
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