96-317-01p

Monsanto

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198
Phone: (314) 694-1000

December 16, 1996

Dr. James Lackey
Botanist, BBEP, APHIS, USDA
4700 River Road Unit 147
Riverdale, MD 20737-1237

Subject: Petition for Determination of Non-Regulated Status:

Insect-Protected Roundup Ready Corn Line MON 802. APHIS Petition No. 96-317-01p.

Roundup Ready Corn Line MON 832. APHIS Petition not yet assigned.

Dear Dr. Lackey:

Monsanto Company recently submitted the two petitions identified above for Insect-Protected/Roundup Ready corn (MON 802) and Roundup Ready corn (MON 832). In a recent telephone conversation with Ms. Shirley Ingebritsen, an inquiry was made as to the corresponding line numbers used for lines MON 802 and MON 832 in USDA Field Trial Termination Reports provided as a part of our submission. In our plant biotech research process at Monsanto all transformation events are initially assigned line numbers. As these events complete various agronomic and technical hurdles, they are assigned MON (Monsanto) numbers to facilitate the various studies which are performed.

The corresponding line number for corn line MON 802 is 599-04-2. The corresponding line number for corn line number MON 832 is 591-03-2.

Should you have any questions, please feel free to contact either Dr. Dickerson at 202-383-2857 or myself (314-537-7488).

Sincerely,

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

cc: Ms. Shirley Ingebritsen

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96-311-01P NOV 12 1996

Monsanto

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198
Phone: (314) 694-1000

November 12, 1996

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

Subject: Petition for Determination of Non-Regulated Status: Insect-Protected Roundup Ready Corn Line MON 802. Monsanto #: 96-134U; Volume I

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) for corn which expresses a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) and is tolerant to applications of the herbicide Roundup®. Field experiments have been conducted since 1993 in the U.S. under United States Department of Agriculture (USDA) notifications as well as an Experimental Use Permit (524-EUP-82) obtained from the EPA in 1994 and renewed in 1995 and 1996. Results from these field experiments have demonstrated that these corn lines are protected season long from the leaf and stalk feeding damage caused by European corn borer (*Ostrinia nubilalis*) and are tolerant to field applications of the herbicide Roundup.

This petition requests a determination from APHIS that insect-protected Roundup Ready corn line MON 802, any progenies derived from crosses between these lines and other corn varieties, and any progeny derived from crosses of these lines with genetically modified corn varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under regulations in 7 CFR part 340.

Enclosed are two original copies of our USDA Petition. An electronic copy of the text portion of this document in a WordPerfect format can be provided for internal USDA editing purposes if desired. Should you have any questions, please feel free to contact either Dr. Dickerson at 202-383-2857 or myself (314-537-7488).

Sincerely,

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

cc: Dr. C.T. Dickerson - Monsanto

96-317-01p
Supplement

Monsanto

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198
Phone: (314) 694-1000

February 18, 1997

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

Subject: Petition for Determination of Non-Regulated Status: Insect-Protected Roundup Ready Corn Line MON 802. USDA Petition 96-317-01p.

Dear Mr. Lidsky:

Monsanto appreciates your letter of January 21, 1996 requesting certain data and information as needed to complete the Environmental Assessment and Determination documents regarding the regulated status of corn line MON 802. This response letter and the attached information is provided as a supplement to the original petition 96-317-01p.

Should you have any questions, please feel free to contact either Dr. Russ Schneider at 202-383-2866 or myself (314-537-7488).

Sincerely,

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

cc: Dr. C.T. Dickerson - Monsanto

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1. **Data (or reference to such) is needed on the expression or lack of expression of *nptII* in the transgenic line MON 802 regardless of the fact that the *nptII* gene is under the control of its bacterial promoter in the transformation vectors.**

In response to this request from the USDA, the following experiment was performed to document the lack of expression of NPTII in line MON 802. A summary of materials and methods and results is included.

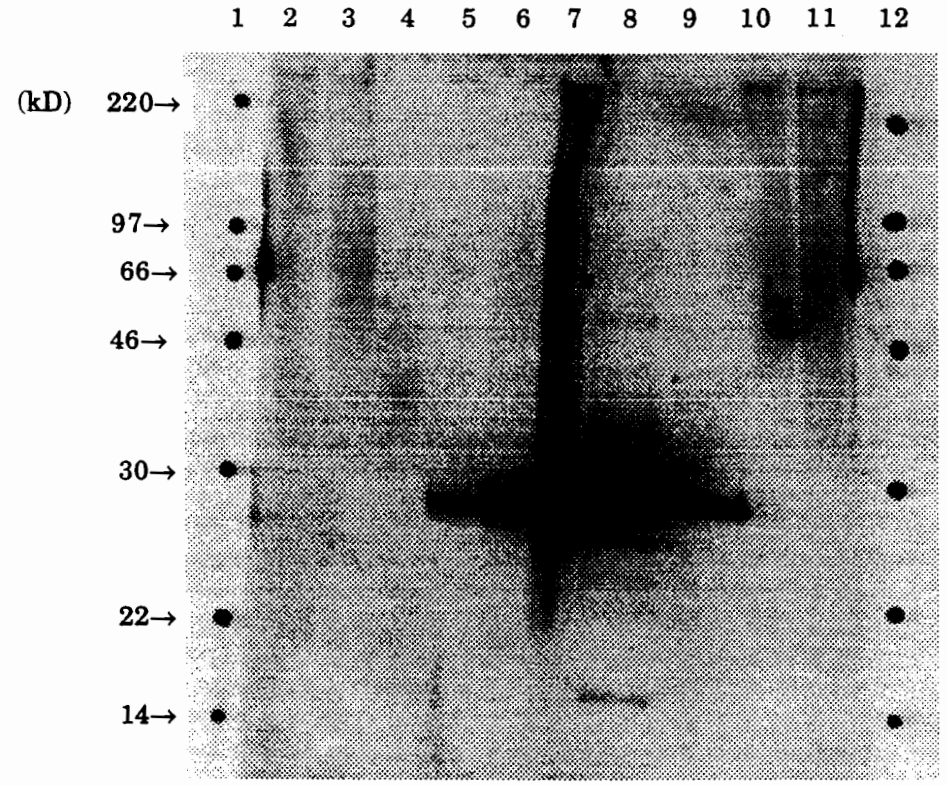
NPTII Western Blot Analysis of Corn Lines MON 802, MON 809, MON 820 and MON 832.

Corn leaf tissue was extracted, using a polytron homogenizer, at a tissue to buffer ratio of 1:50 with SDS grinding buffer (0.07 mM SDS, 6.26 mM Tris, and 10% glycerol). NPTII standard (lot# 5524618), at a concentration of 1.084 mg/mL, was appropriately diluted to load 25 pg, 100 pg and 1 ng and spiked into plant matrix. 25 pg and 1 ng NPTII protein standard were also loaded without matrix. Molecular weight markers were loaded on the gels to approximate the size of the protein band. Twenty microliters of leaf extract were loaded onto each lane of a 10-18% Tris-Glycine gel and proteins were separated by SDS-PAGE at 35 mA/gel.

The proteins were electroblotted to PVDF membrane and blocked for two days with 10% non-fat dry milk/TBST at 4°C. The blot was incubated for one hour with NPTII monoclonal primary antibody #7 from mouse (lot# M355-E2-A2-A2) at a 1:2500 dilution in 2% non-fat dry milk/TBST. The blot was washed and incubated for 45 minutes with anti-mouse serum conjugated to HRP (Promega) in 2% non-fat dry milk/TBST. The blot was rinsed and developed using Enhanced-chemiluminescence kit (Amersham). The blot was exposed to X-Omat film (Kodak) for 3 minutes. Lanes 1 and 12 (containing the rainbow markers) were removed following transfer and were aligned with the developed film and marked for the scan.

The Western blot results are presented in the accompanying figure. The limit of detection for the NPTII protein in plant matrix is approximately 25 pg (lane 5). The NPTII protein band at ~29 kD was not detected in leaf extracts of MON 802 (lane 10), MON 809 (lanes 2 and 3), MON 820 (lane 4) and MON 832 (lane 11) corn lines. This result confirms the lack of expression of NPTII from corn transformation plasmids containing the NPTII gene regulated by a bacterial promoter.

Western Blot Analysis of NPTII Protein of Insect-Protected Corn Line MON 809, Insect-Protected Roundup Ready Corn Line MON 802 and Roundup Ready Corn Line MON 832[1]



Lane	
1	Amersham High-Range Rainbow Markers
2	MON 809 Extract: (Study #: 96-02-50-02 Sample ID# 80941)
3	MON 809 Extract: (Study #: 95-01-50-01 Sample ID# 80972)
4	MON 820 Extract: (Study #: 95-01-50-01 Sample ID# 82082)
5	25 pg NPTII Standard Spiked into MON 820 Matrix
6	100 pg NPTII Standard Spiked into MON 820 Matrix
7	1 ng NPTII Standard Spiked into MON 820 Matrix
8	1 ng NPTII Standard
9	25 pg NPTII Standard
10	MON 802 Extract: (Study #: 95-01-50-01 Sample ID# 80272)
11	MON 832 Extract: (Study #: 95-01-50-01 Sample ID# 83282)
12	Amersham High-Range Rainbow Markers

[1]: 20 µL leaf extract was run through SDS-PAGE gel, blotted to PVDF membrane, probed with monoclonal primary antibody/monoclonal:HRP secondary antibody and developed with ECL detection kit.

2. **Please clarify the identity of MON 818, first mentioned on page 39 of the petition. Is this corn line synonymous with the recipient corn tissue High Type II?**

The corn plant tissue used in corn transformation was a High Type II genetic material. Genetically comparable control corn was generated by crossing High Type II plants to B73, and additional crossing to Mo17, to produce MON 818 grain. MON 818 as the appropriate control corn line in this study was Mo 17 X (Hi-II X B73) genotype. MON 818 did not contain the genes encoding the CryIA(b), CP4 EPSPS or GOX proteins.

3. **Molecular weight size markers are needed on the Southern blots in order to judge the ability of the gels to resolve restriction fragments that could be of similar size, particularly those of high molecular weight (e.g., those above 23 kb).**

In order to determine high molecular weight sizes of DNA on Southern blots, the gels were made with 0.6% agarose and with 0.5 μg ethidium bromide per ml. and then were electrophoresed for about 40hrs at about 22 v., constant voltage. After the DNA had been electrophorese, a Polaroid picture was taken of the gel with a ruler laid next to the molecular weight markers (Figure 1). The DNA in the gels was transferred to Zeta-Probe® membrane. After the transfer was complete, the molecular weight markers were cut off the membrane. The membrane was then probed with the appropriate ^{32}P labelled probe. The membrane was then exposed to X-ray film. Because there was some residual bacterial DNA in the probes, the molecular weight markers whose source is bacterial DNA tended to hybridize with the probe and was detected on the film. When this occurred, the bands were very intense due to hybridizing with the ^{32}P labelled probe (Figure 2).

The size of the plant DNA bands that hybridized to the ^{32}P probe, when no markers were present on the membrane, was determined by measuring the distance the band had migrated from the top of the gel. This was then correlated it to the molecular weight marker at the same size measurement. When the band and marker did not line up precisely, an estimate of the size was performed to determine the size of the plant DNA band.

These techniques and the figures provided are representative for the determination of high molecular weight sizes of DNA on Southern blots.

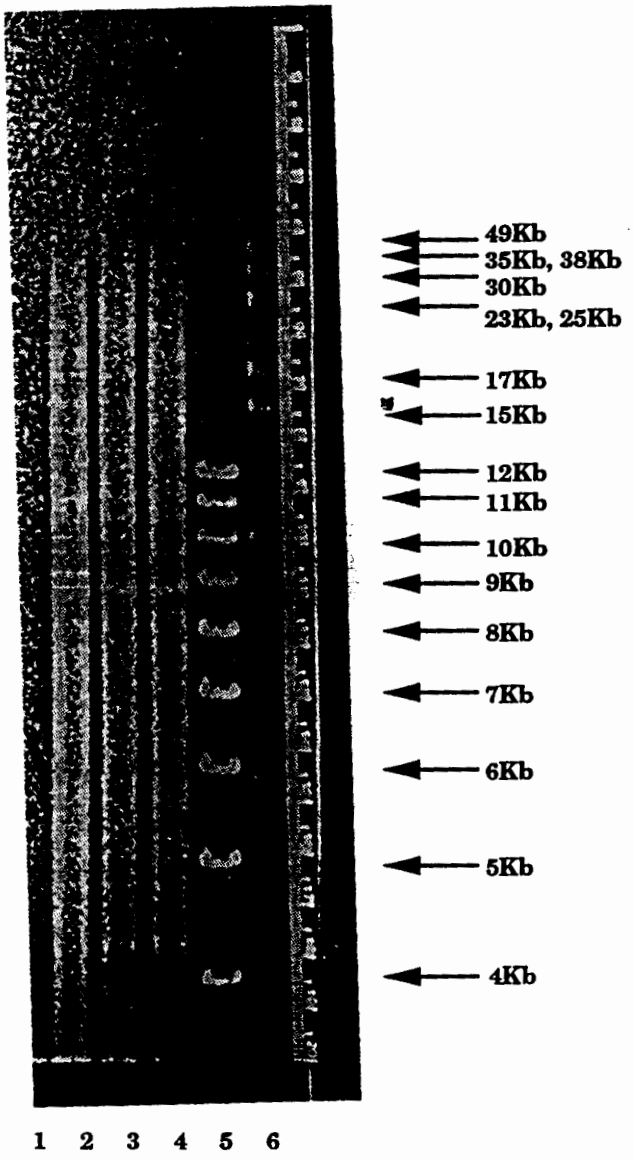


Figure 1. Ethidim bromide stained gel of ^{32}P HSP70 intron Southern blot. Lane 1: PV-ZMGT03 cut with HindIII; lane 2: MON 818 cut with NdeI; lane 3: MON 802 cut with NdeI; lane 4: MON 805 cut with NdeI; lane 5: 1Kb molecular weight marker; lane 6: high molecular weight marker. The sizes of the markers are shown to the right of the markers. Both markers have a 10Kb band and they line up with each other. The gel was blotted to Zeta-Probe membrane; the markers were then cut off the membrane.

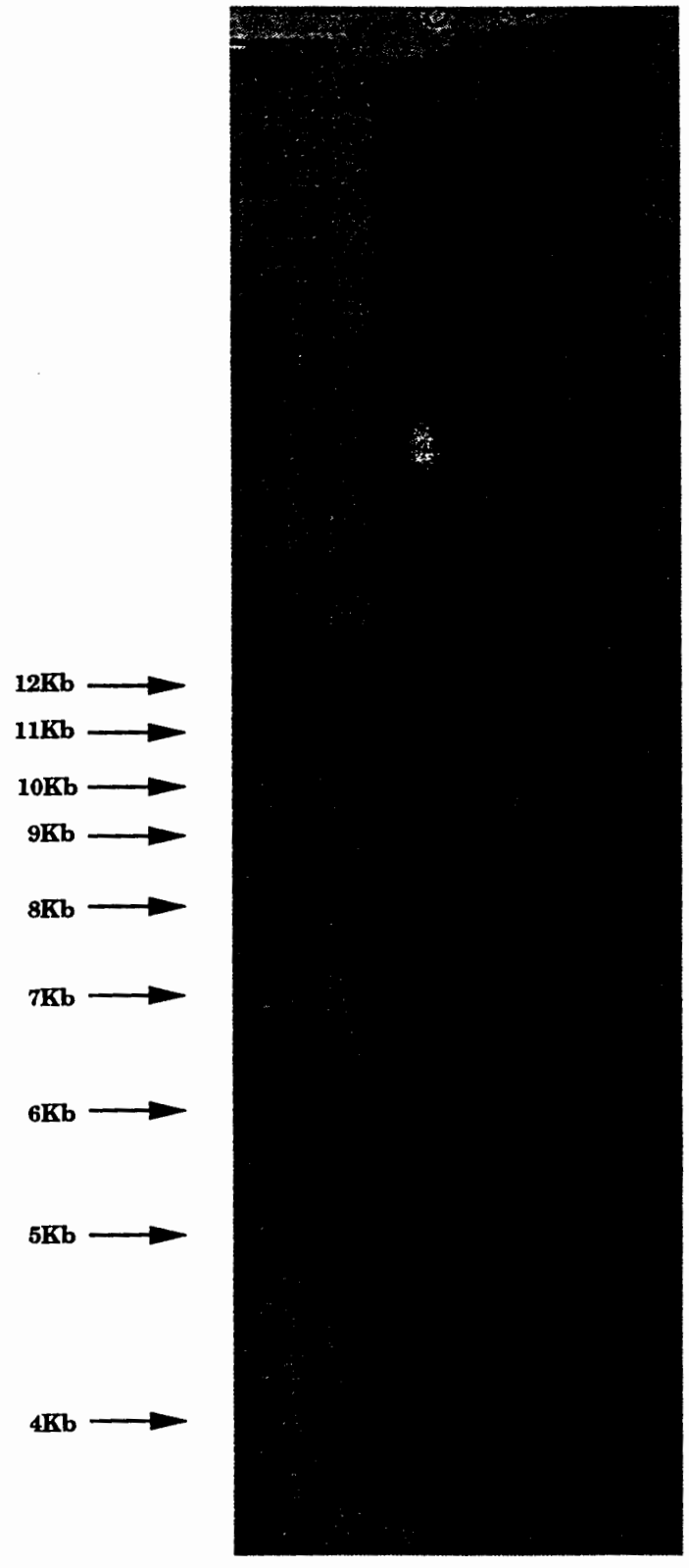


Figure 2. Southern blot of Figure 1 in a mirror image orientation. Lane 1: PV-ZMGT03 cut with HindIII; lane 2: MON 818 cut with NdeI; lane 3: MON 802 cut with NdeI; lane 4: MON 805 cut with NdeI; lane 5: 1Kb moleculare weight marker. Not all of the marker was removed when the membrane was cut. Therefore the probe hybridized to the remaining part of the marker. The sizes of the markers are shown to the left of the markers.

4. **A more thorough description of the probes used for the Southern blots is needed. For example, do they include all or a portion of the coding regions for the genes, and do they include the promoters, terminators, or an intron? Which restriction fragments contain the probe sequence?**

Probes homologous to the cryIA(b), CP4 EPSPS, gox, nptII coding regions, and ori-pUC element were prepared by polymerase chain reaction (PCR) or restriction enzyme digestions using the appropriate plasmid (PV-ZMBK15 and PV-ZMGT03). The plasmid restriction fragments used as probes are identified below.

Table 1.0 Table of plasmid restriction fragments used as probes for molecular analysis of MON 802

Elements	PV-ZMGT03	PV-ZMBK15
gox	NcoI/EcoRI 1302bp	
nptII	BglII/NcoI 596bp	
cryIA(b)		NcoI/EcoRI 3464bp
CP4 EPSPS	SphI*/EcoRI 1357bp	
ori pUC	AflIII/AluNI^ 416bp	

* : SphI site is at bp 1818, between CTP2 and CP4 EPSPS coding region
^ : AflIII site is at bp 7349; AluNI site is at bp 7765

96-317-01p

Petition for Determination of Nonregulated Status:

Insect-Protected Roundup Ready Corn Line MON 802

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

Submitted by:

Kent A. Croon, Regulatory Affairs Manager
Monsanto Company
700 Chesterfield Parkway North, BB3A
Chesterfield, MO 63198
Tel: 314-537-7488
Fax: 314-537-7085

November 15, 1996

#96-134U
Volume I (MON 802)

Prepared by:

Kent A. Croon, Pat R. Sanders and Jean-Noel Mutz

Contributors:

R. Fuchs, R. Buehler

Petition For Determination of Nonregulated Status for Insect-Protected Roundup Ready™ Corn Line MON 802

Summary

Monsanto Company is submitting this Petition for Determination of Non-regulated Status to the Animal Plant Health Inspection Service (APHIS) for corn which expresses a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) and is tolerant to applications of the herbicide Roundup®. This petition requests a determination from APHIS that insect-protected Roundup Ready corn line MON 802, any progenies derived from crosses between this line and other corn varieties, and any progeny derived from crosses of this line 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.

Insect-Protection

Corn is the largest crop in the United States in terms of planted acreage, total production, and crop value. United States production in 1993 was 161 million metric tons produced on over sixty million acres with the majority of national production concentrated across what is known as the "Corn Belt" in the upper Midwest. The European corn borer (ECB), *Ostrinia nubilalis*, causes severe economic damage as it feeds on leaf and stalk tissue compromising the structural integrity of the corn plant. This feeding damage leads to plant lodging and yield loss. Chemical insecticides offer limited utility as applications must be made prior to the time the insect bores into the stalk and repeat applications are often necessary. As one of the most important pests of corn in the United States, it is estimated that ECB causes an average five to ten percent crop production loss annually in corn.

Monsanto has developed genetically modified corn plants that effectively control ECB. These genetically modified corn plants produce an insect control protein (CryIA(b)) derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). Microbial formulations containing these insecticidal proteins have been registered by the Environmental Protection Agency (EPA) and commercially available for over thirty years. The protein produced by insect-protected corn is identical to that found in nature and in commercial *B.t.k.* formulations registered as pesticides with the EPA. This protein is highly selective in controlling ECB and is expressed at an effective level in plant tissue throughout the growing season.

Field experiments were conducted from 1993 through 1995 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 by the Agency in 1995 and 1996.

Results from these field experiments have demonstrated that insect-protected corn is protected season long from the leaf and stalk feeding damage caused by ECB. Growers planting insect-protected corn will not require insecticide applications to control 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 insect-protected 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, 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. The use of insect-protected 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 targeted lepidopteran insect pests including 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.
- A potential reduction in the occurrence of ear rots associated with ECB damage to the corn ear and associated production of harmful mycotoxins.

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

The consistent control afforded by insect-protected Roundup Ready corn line MON 802 will enable growers to significantly reduce the amount of chemical pesticides now applied to their crop for control of ECB while maintaining yield potential. In addition, 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 ECB. 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.

Roundup Tolerance

The use of Roundup Ready corn will provide farmers new options for effective weed control. Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate has excellent environmental features, such as rapid soil binding (resistance to leaching) and biodegradation (which decreases persistence), as well as extremely low toxicity to mammals, birds and fish.

The use of corn plants containing the Roundup Ready genes for corn production would enable the farmer to utilize Roundup herbicide for effective control of weeds during the growing season and to take advantage of this herbicide's environmental and safety characteristics. Roundup Ready corn can positively impact current agronomic practices in corn by:

- Offering the farmer a new, wide-spectrum weed control option.
- Allowing the use of an environmentally acceptable herbicide.
- Providing a novel herbicide for in-season corn weed control.
- Increasing flexibility to treat weeds on an "as needed" basis.
- Providing an excellent fit with reduced-tillage systems, which results in increased soil moisture, while reducing soil erosion and fuel use.
- Providing cost-effective weed control.

Therefore, Monsanto Company requests a determination from APHIS that insect-protected Roundup Ready corn line MON 802, any progenies derived from crosses between this line and other corn varieties, and any progeny derived from crosses of this line 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.

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.

Kent A. Croon, Regulatory Affairs Manager
Monsanto Company
700 Chesterfield Parkway North, BB3A
Chesterfield, MO 63198
Tel: 314-537-7488
Fax: 314-537-7085

Abbreviations Used in this Petition for the Determination of Non-Regulated Status of Insect-protected Roundup Ready Corn Line MON 802

2,4-D	(2,4-dichlorophenoxy)acetic acid
APHIS	Animal Plant Health Inspection Service
B.t.k.	*Bacillus thuringiensis* subsp. *kurstaki*
CaMV	Cauliflower mosaic virus
CFR	Code of federal regulations
CP4 EPSPS	EPSPS from *Agrobacterium* sp. strain CP4
cryIA(b)	Class I (Lepidoptera-specific) crystal protein gene
CTP	Chloroplast transit peptide
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
E35S	35S promoter with enhancer sequence
ECB	European corn borer
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
EUP	Experimental Use Permit
FDA	Food and Drug Administration
FFDCA	Federal Food Drug and Cosmetic Act
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
Ga, ga	Gametophyte
GDD	Growing degree days
GLP	Good Laboratory Practice
gox	Gene for glyphosate oxidase
GOX	Glyphosate oxidase
*hsp*70	Intron sequence from maize heat-shock protein 70
IPM	Integrated Pest Management
kD	Kilodaltons
M	Million
ml, l	Milliliter, liter
mm, cm, m	millimeter, centimeter, meter
ng, µg, mg, g, kg	Nanogram, microgram, milligram, gram, kilogram
NOS3'	3' transcriptional termination sequence from nopaline synthase
NPTII	Neomycin phosphotransferase II
nptII	Gene for neomycin phosphotransferase II
*ori-p*UC	Bacterial origin of replication from the pUC plasmid
PCR	Polymerase chain reaction
ppm	Part per million
sp	Species
subsp.	Subspecies
USDA	United States Department of Agriculture

Petition for Determination of Nonregulated Status of Insect-Protected Roundup Ready Corn Line MON 802

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Appendices

Part I. Rationale for Development of Insect-Protected Roundup Ready Corn

A. Benefits of Insect-Protected Corn

Corn is the largest crop in the United States in terms of planted acreage, total production, and crop value (National Corn Growers Association, 1995). United States production in 1994 was estimated at 256 million metric tons produced on nearly 73 million acres with the majority of national production concentrated across what is known as the "Corn Belt" in the upper Midwest. The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), causes severe economic damage as it feeds on leaf and stalk tissue compromising the structural integrity of the corn plant (Dicke and Guthrie, 1988; Cooperative Extension Service, 1989). This feeding damage leads to plant lodging and yield loss. Chemical insecticides offer limited utility as applications must be made prior to the time the insect bores into the stalk and repeat applications are often necessary. As one of the most important pests of corn in the United States, it is estimated that ECB causes an average five to ten percent crop production loss annually in corn with potentially greater losses in areas of high infestation (Bergman *et al.*, 1985a-f; Bode and Calvin, 1990; Briggs & Guse 1986; Guthrie *et al.*, 1975; Rice, 1994a-c).

Monsanto has developed genetically modified corn plants that effectively control ECB throughout the growing season. These genetically modified corn plants produce an insect control protein (CryIA(b)) derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). The CryIA(b) protein produced in Insect-protected Roundup Ready corn line MON 802 is identical to CryIA(b) protein found in nature and in commercial microbial formulations (EPA MRID no. 43533203). Microbial formulations containing these insecticidal proteins have been registered by the EPA and commercially available since 1961 (EPA, 1988; Lüthy *et al.*, 1982).

The CryIA(b), CP4 EPSPS and GOX proteins are expressed at low levels in corn leaf, whole plant and grain tissues. Environmental fate studies have shown that the CryIA(b) protein is rapidly degraded in the soil and does not persist in the environment (EPA MRID no. 43533206). In addition, the risk of an uncontrolled introduction of the genes through hybridization or outcrossing to a native species is virtually nonexistent in the United States, as no wild relatives grow with which corn can cross (Croon *et al.*, 1995).

Results from field experiments conducted from 1993 through 1995 throughout the corn growing regions have demonstrated that insect-protected corn line MON 802 is protected season long from the leaf and stalk feeding damage caused by ECB. Growers planting insect-protected corn will not require insecticide applications to control this pest. This reduction in insecticide use will enhance biological control and the implementation of other pest

management strategies for other corn pests not susceptible to the CryIA(b) protein such as spider mites and aphids.

In support of Monsanto's request to the EPA for the registration and exemption from the requirement of a tolerance for the CryIA(b) protein as a plant pesticide, studies demonstrating the safety of this protein to nontarget organisms were conducted. These studies demonstrated that the CryIA(b) protein has a limited spectrum of insecticidal activity with no deleterious effect on beneficial insects, mammals, or birds (EPA MRID nos. 43468002 through 43468005, 43439202, 43439203, 43468001 and 43533205). These results fully confirm the findings of similar studies conducted with commercially available microbial *B.t.* formulations.

The commercialization of insect-protected Roundup Ready corn line MON 802 (and any progenies derived from crosses to other corn varieties) following receipt of all required approvals, will represent an efficacious and environmentally compatible addition to the existing options for corn insect pest management. The use of insect-protected 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 targeted lepidopteran insect pests including 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.
- A potential reduction in the occurrence of ear rots associated with ECB damage to the corn ear and associated production of harmful mycotoxins.
- Both large and small growers will benefit from the planting of insect-protected corn as no additional labor, planning, or machinery is required.

In summary, the consistent control afforded by insect-protected Roundup Ready corn line MON 802 will enable growers to significantly reduce the amount of chemical pesticides now applied to their crop for control of ECB while maintaining yield potential. In addition, 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 ECB. 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. A discussion of the agronomic benefits of insect-protected corn as prepared by Dr. Kevin Steffey, Entomologist at the University of Illinois, has been previously provided (Croon *et al.,* 1995).

B. Benefits of Roundup Ready Corn

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #'s 1071-83-6, 38641-94-0), the active ingredient in the non-selective, foliar-applied, broad-spectrum, post-emergent herbicide Roundup (Baird, 1971; Malik *et al.,* 1989), is the world's most popular herbicide. This is primarily due to its excellent weed control capabilities and its well-known, favorable environmental and safety characteristics. However, the sensitivity of crop plants to glyphosate has prevented the in-season use of this herbicide over-the-top on crops. The extension of the use of Roundup herbicide to allow in-season application in major crops such as corn will provide new weed control options for farmers. Recent advances in plant biotechnology have made it possible to insert genes to provide crop tolerance specifically to the non-selective herbicide glyphosate, and bring the benefits of its use to weed management in corn (Padgette *et al.,* 1996).

Weed management is a critical step to maximize corn yields and retain a high-quality harvest, free of weed seeds. For effective weed control, the farmer typically selects a herbicide based on several factors: weed spectrum, lack of crop injury, cost and environmental characteristics. Few herbicides available today deliver optimal performance in all of these areas. Several classes of herbicides are effective for broad-spectrum weed control, but many are either non-selective and kill crop plants or they significantly injure some crops at the application rates required for effective weed control.

The use of Roundup Ready corn will provide farmers new options for effective weed control. Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate has excellent environmental features, such as rapid soil binding (resistance to leaching) and biodegradation (which decreases persistence), as well as extremely low toxicity to mammals, birds and fish (Malik *et al.,* 1989). Recently, glyphosate was classified by the EPA as Category E (evidence of non-carcinogenicity for humans) (57 FR 8739). Studies separate from those summarized herein have

been provided to the EPA in a request to amend the Roundup herbicide label to include in-season application on Roundup Ready corn.

The use of corn plants containing the Roundup Ready genes for corn production would enable the farmer to utilize Roundup herbicide for effective control of weeds during the growing season and to take advantage of this herbicide's environmental and safety characteristics. Roundup Ready corn can positively impact current agronomic practices in corn by:

- Offering the farmer a new, wide-spectrum weed control option.
- Allowing the use of an environmentally acceptable herbicide.
- Providing a novel herbicide for in-season corn weed control.
- Increasing flexibility to treat weeds on an "as needed" basis.
- Providing an excellent fit with reduced-tillage systems, which results in increased soil moisture, while reducing soil erosion and fuel use.
- Providing cost-effective weed control.

C. Regulatory Approvals

Before commercializing insect-protected Roundup Ready corn line MON 802, Monsanto will seek the following regulatory approvals:

1. This determination from USDA/APHIS that insect-protected Roundup Ready MON 802, and all progenies from crosses between this line 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 insect-protected corn under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

3. An exemption from the requirement of a tolerance for the CryIA(b) insecticidal protein, the CP4 EPSPS and GOX selectable marker enzymes, and the genetic material necessary for the production of these proteins in plants under sections 408 of the Federal Food Drug and Cosmetic Act (FFDCA) from the EPA. Tolerance exemptions for the CryIA(b) and CP4 EPSPS proteins in plants were granted on August 2, 1996 (FR 61:150 pgs. 40340-40343; FR 61:150 pgs. 40338-40340). A request for an exemption from the requirement of a tolerance for the GOX selectable marker is currently under review by the Agency.

4. Registration of Roundup herbicide (EPA Reg. No. 524-445) for use over-the-top of Roundup Ready corn. This application has been previously submitted to the EPA.

In addition, we have completed our consultation on insect-protected Roundup Ready corn line MON 802 which was 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.

D. References

Baird, 1971. Introduction of a New Broad Spectrum Postemergence Herbicide Class with Utility for Herbaceous Perennial Weed Control. *North. Cent. Weed Control Conf.* 26:64-68.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985a. Pesticide assessment of field corn and soybeans: Corn Belt states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 26 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985b. Pesticide assessment of field corn and soybeans: Delta states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 28 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985c. Pesticide assessment of field corn and soybeans: Lake states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 22 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985d. Pesticide assessment of field corn and soybeans: Northeastern states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 28 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985e. Pesticide assessment of field corn and soybeans: Northern Plains states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 22 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985f. Pesticide assessment of field corn and soybeans: Southeastern states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 26 pp.

Bode, W.M. and D.D. Calvin. 1990. Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. J. Econ. Entomology. 83(4): 1595-1603.

Briggs, S.P. & C.A. Guse. 1986. Forty years of European corn borer data: what have we learned? pp. 169-173. *In* Thirty-Eighth Illinois Custom Spray Operators Training Manual, Coop. Ext. Serv., Univ. of Illinois, Urbana-Champaign.

Cooperative Extension Service. 1989. European Corn Borer Development and Management. North Central Regional Extension Publication No. 327. Extension Service, U.S.D.A., Washington, D.C.

Croon, K.A., P.R. Sanders, P.J. Keck, T.C. Lee, and G.B. Parker. 1995. Petition for Determination of Nonregulated Status: Insect-protected Corn (Zea mays L.) with the cryIA(b) Gene from Bacillus thuringiensis subsp. kurstaki. USDA Petition no. 95-093-01p.

Dicke, F.F. and W.D. Guthrie. 1988. The Most Important Corn Insects. In Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin.

EPA. 1988. Guidance for the registration of pesticide products containing Bacillus thuringiensis as an active ingredient. NTIS PB 89-164198.

EPA MRID no. 43439202. Maggi, V.L. and S.R. Sims. 1994. "Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Larvae", Study Number IRC-91-ANA-13, an unpublished study conducted by Monsanto Company and California Agricultural Research, Inc.

EPA MRID no. 43439203. Maggi, V.L. and S.R. Sims. 1994. "Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Adults", Study Number IRC-91-ANA-12, an unpublished study conducted by Monsanto Company and California Agricultural Research, Inc.

EPA MRID no. 43468001. Naylor, M. 1992. "Acute Oral Toxicity Study of *Btk* HD-1 Tryptic Core Protein in Albino Mice".

EPA MRID no. 43468002. Sims, S.R. 1994. "Stability of the CryIA(b) Insecticidal Protein of Bacillus thuringiensis var. kurstaki (B.t.k. HD-1) in Sucrose and Honey Solutions Under Non-Refrigerated Temperature Conditions", Study Number IRC-91-ANA-11, an unpublished study conducted by Monsanto Company.

EPA MRID no. 43468003. Hoxter, K.A. and S.P. Lynn. 1992. "Activated Btk HD-1 Protein: A Dietary Toxicity Study with Green Lacewing Larvae", Study Number WL-92-155, an unpublished study conducted by Monsanto Company and Wildlife International Ltd.

EPA MRID no. 43468004. Hoxter, K.A. and S.P. Lynn. 1992. "Activated Btk HD-1 Protein: A Dietary Toxicity Study with Parasitic Hymenoptera (Brachymeria intermedia)", Study Number WL-92-157, an unpublished study conducted by Monsanto Company and Wildlife International Ltd.

EPA MRID no. 43468005. Hoxter, K.A. and S.P. Lynn. 1992. "Activated Btk HD-1 Protein: A Dietary Toxicity Study with Ladybird Beetles", Study Number WL-92-156, an unpublished study conducted by Monsanto Company and Wildlife International Ltd.

EPA MRID no. 43533203. Lee, T.C., Bailey, M., and Sanders, P.R. "Compositional Comparison of Bacillus thuringiensis subsp. kurstaki HD-1 Protein Produced in ECB Resistant Corn and the Commercial Microbial Product, DIPEL®", Study Number 94-01-39-12, an unpublished study conducted by Monsanto Company.

EPA MRID no. 43533205. Campbell, S.M., and Beavers, J.B. "A Dietary Toxicity Study with MON 80187 Meal in the Northern Bobwhite", Project Number 139-387, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto Company.

EPA MRID no. 43533206. Sims, S.R., and Sanders, P.R. "Aerobic Soil Degradation of Bacillus thuringiensis var. kurstaki HD-1 Protein", Study Number 94-01-39-13, an unpublished study conducted by Monsanto Company.

Guthrie, W.D., W.A. Russell, F.L. Neumann, G.L. Reed, and R.L. Grindeland. 1975. Yield losses in maize caused by different levels of infestation of second-brood European corn borers. Iowa State Journal of Research. Vol. 50, no. 2.

Lüthy, P., J.L. Cordier and H.M. Fischer. 1982. Bacillus thuringiensis as a bacterial insecticide: Basic considerations and application. In Microbial and Viral Pesticides (E. Kurstak, ed.), pp. 35-71, Marcel Dekker, Inc., New York.

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

National Corn Growers Association. 1995. The World of Corn. St. Louis, Missouri, USA.

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

Rice, M.E. 1994a. Aerial application of insecticides for second generation European corn borer control 1993. p. 206. *In* Arthropod Management Tests: 1994. Entomological Society of America, Lanham, Maryland.

Rice, M.E. 1994b. Second generation European corn borer control with aerial application of insecticides. 1992. pp. 205-206. *In* Arthropod Management Tests: 1994. Entomological Society of America, Lanham, Maryland.

Rice, M.E. 1994c. Aerial application of insecticides for control of second generation European corn borer. 1991. p. 204-205. *In* Arthropod Management Tests: 1994. Entomological Society of America, Lanham, Maryland.

Part II. The Corn Family

Potential for Outcrossing and Weediness of Genetically Modified Insect-protected Corn

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Introduction

Corn (*Zea mays* L.), or maize, is one of the few major crop species indigenous to the Western Hemisphere. 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. The putative parents of corn have not been recovered, but it seems teosinte probably played an important role in the genetic background of corn. 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. 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, and life cycle of corn is presented, followed by a discussion of how the characteristics of cultivated corn affect gene flow between cultivated corn and its wild relatives.

A. The Corn Family

1. History of corn

Corn originated in the highlands of Mexico 7,000 to 10,000 years ago. By the time Columbus discovered the Western Hemisphere, corn was being grown by the indigenous civilizations from Chile to southern Canada. Columbus noted the presence of corn on the north coast of Cuba November 5, 1492 and introduced corn to Europe upon his return to Spain (Goodman, 1988). After the introduction of corn to Europe, corn became distributed within two generations throughout the world where it could be cultivated. Today, corn ranks third after wheat and rice as one of the world's three leading food crops. Unlike wheat and rice, more corn is consumed by livestock rather than directly by humans. Corn, however, is consumed directly by humans in the tropics and in the Southern Hemisphere.

The original corn growing areas did not include the north-central area (U.S.Corn Belt) of the United States. The highly productive U.S. Corn Belt dent corns were derived after the colonization of North America. The European settlers accepted the local native American varieties and

incorporated them with other crops to provide food, feed, and fuel for their survival. The current U.S. Corn Belt dent corns evolved from the gradual mingling of those settlements that spread north and west from the southeastern North America and those settlements that spread south and west from the northeastern North America.

The corns grown in the northeast are called northern flints; their origin is not clear, but races from the highlands of Guatemala have similar ear morphology (Goodman and Brown, 1988). Northern flints are largely eight-rowed with cylindrical ears, are early maturing, and are short statured plants with tillers. The southern dent corns grown in the southeast United States seemed to have originated from the southeast coast of Mexico. Southern dent corns are characterized as having tall, late maturing, non-tillered, poorly rooted plants with soft-textured white kernels on many rowed, tapering ears. It seems the Tuxpeno race contributed to the development of southern dents. The intentional and/or unintentional crossing between the early northern flints and late southern dents led eventually to the highly productive U.S. Corn Belt dent corns that are used extensively throughout the world today.

The origin of corn has been studied extensively, and hypotheses for the origin and for the parentage of corn have been advanced (Mangelsdorf, 1974). Hypotheses suggested for origin of corn include the following: 1) cultivated corn is a descendent of pod corn; 2) corn originated by direct selection from teosinte; 3) corn, teosinte, and *Tripsacum* descended independently from a common, unknown ancestor; and 4) the tripartite theory: a) corn originated from pod corn, b) teosinte derived from a cross of corn and *Tripsacum*, and c) modern corn varieties evolved by corn intercrossing with teosinte or *Tripsacum* or both (Mangelsdorf, 1974).

It has been suggested that modern corn originated from corn grass by a single-gene mutation causing ear development. Other suggestions have included *Coix* and species of the genus *Manisuris* in the tribe *Andropogoneae* for contributing to the genome of corn. The hypotheses have been tested by the study of crosses for genome commonality, fertility, variation, and segregation of morphological plant traits, by archeological evidence, and by use of molecular genetic markers.

Evidence has been reported to support the different hypotheses, but it seems the preponderance of evidence supports the hypothesis that corn descended from teosinte (Galinat, 1988). The teosinte genome is similar to corn, teosinte easily crosses with corn, and teosinte has several plant morphological traits similar to corn. Teosinte has a more weedy appearance and more tillers than modern corn varieties. The one major distinguishing difference between corn and teosinte is the female inflorescence, or ear. Modern corn varieties have 1 to 3 lateral branches that terminate in an ear with 8 to 24 kernel rows of 50 seeds, and the ear is enclosed in modified leaves or husks. Teosinte also has lateral branches, but they terminate in two-rowed spikes of perhaps 12 fruit

cases, with each fruit case having one seed enclosed by an indurated glume (Goodman, 1988).

2. Taxonomy of the genus *Zea*

Corn is a member of the tribe Maydae, which is included in the subfamily Panicoideae of the grass family Gramineae (Table II.1). The genera included in the tribe Maydae include *Zea* and *Tripsacum* in the Western Hemisphere and *Coix*, *Polytoca*, *Chionachne*, *Schlerachne*, and *Trilobachne* in Asia. Although the Asian genera have been implicated by some in the origin of corn, the evidence for them is not as extensive and convincing as for the genera located in the Western Hemisphere.

There has been some fluctuation in Latin binomial designations of the species included in *Zea* in recent years and the classification will be used herein (Doebley and Iltis, 1980).

The genus *Zea* includes two subgenera: *Luxuriantes* and *Zea*. Corn (*Zea mays* L.) is a separate species within the subgenus *Zea* along with three subspecies. All of the species within the genus *Zea*, except corn, are different species of teosinte. Until recently, the teosinte species were included in the genus *Euchlaena* rather than the genus *Zea*.

The other genus included in the Maydae tribe is *Tripsacum*. *Tripsacum* includes 16 species with a basic set of 18 chromosomes (n = 18), and the different species of *Tripsacum* include multiples of 18 chromosomes ranging from 2n = 36 to 2n = 108.

Five genera are included in the tribe Maydeae that originated in Asia. Except for *Coix*, the basic chromosome number is n = 10. Within *Coix*, n = 5 and n = 10 have been reported.

3. Genetics of corn

Corn is genetically one of the best developed and best characterized of the higher plants. Because of the separation of male and female inflorescence, number of seeds produced on female inflorescence, ease in handling (growing and hand pollinating), nature of the chromosomes, and low basic chromosome number (n = 10), corn has been accessible for study at all levels of genetics. Corn was one of the first crop species included in genetic laboratories to obtain a basic understanding of mitosis, meiosis, chromosome segregation, linkage and effects of crossing-over, and transposable elements. Because of the importance of corn in the U.S. and world economies, and the genetic information obtained since 1900, corn has continued to receive extensive study in modern genetic laboratories.

Molecular geneticists have developed extensive genetic maps of corn to complement the genetic maps developed by the early corn geneticists. Corn has been used in tissue culture research, in extensive studies to relate molecular markers to qualitative and quantitative traits, in sequencing of

genes, in study of transposable elements for gene tagging and generating genetic variability, in gene transformation, etc.

Extensive compilations on corn genetics, corn cytogenetics, cell tissue culture, and on molecular genetics were provided (Coe *et al.,* 1988; Carlson, 1988; Phillips *et al.,* 1988; Walbot and Messing, 1988). Rapid advances are being made daily in corn genetics, but these are useful references.

4. Life cycle of corn

Corn is an annual and the duration of the life cycle depends on the cultivars and on the environments in which the cultivars are grown (Hanway, 1966). Corn cannot survive temperatures below 0° C (32° F) for more than 6 to 8 hours after the growing point is above ground (5 to 7 leaf stage). Damage from freezing temperatures, however, depends on the extent of temperatures below 0° C, soil condition, residue, length of freezing temperatures, wind movement, relative humidity, and stage of plant development. Light frosts in the late spring of temperate areas can cause leaf burning, but the extent of the injury usually is not great enough to cause permanent damage, although the corn crop will have a ragged appearance because the leaf areas damaged by frost persist until maturity. The completion of the life cycle of corn, therefore, is dictated by the duration of the average number of frost-free days.

The number of frost-free days dictates the corns with differences in length of their life cycles be grown in north-to-south directions of temperate areas. In the United States, corns with relative maturities of 80 days or less are grown in the extreme northern areas, and corns with relative maturities of more than 125 days are grown in the southern areas. Corns having relative maturities of 100 to 115 days are typically grown in the U.S. Corn Belt. Relative maturities, however, are not parallel lines east-to-west because they are dependent on prevailing weather patterns, topography, large bodies of water, and soil types (Troyer, 1994).

Another measure used to judge the relative maturities of corns is the number of growing degree days (GDD) required from emergence to maturity. Based on GDD required to mature, corns are assigned to areas that have, on the average, less than 1850 GDD in the extreme northern areas of the United States to corns that require more than 2750 GDD in more southern areas. Assume a 115-day maturity hybrid is grown in central Iowa. Average last frost date is May 1 and average first frost date is October 5, resulting in an expected 158 frost-free days. If average emergence is May 15 and average flowering is July 15, 60 days are required from emergence to flowering. Corn requires 50 to 60 days to attain physiological maturity. If physiological maturity occurs 55 days after flowering, physiological maturity will occur on or about September 10, or 115 days from emergence to physiological maturity.

If one considers the central U.S. Corn Belt as an example, the following time-frame for each stage of corn development could be as follows:

Planting date: May 1 ± 10 days
Date emergence: May 10 ± 4 days
Date of flower: July 20 ± 10 days
Physiological maturity: September 10 ± 5 days
Harvest maturity: October 10 ± 10 days

These suggested time frames can vary within the same year among locations and among years at the same location, depending on the environmental conditions experienced from planting to harvesting.

5. Hybridization

Hybridization is a fundamental concept used in the breeding, production, and growing of corn in the United States. Corn evolved as an open-pollinated (cross-fertilizing) crop species and until the 20th century the corn cultivars were what we designate today as open-pollinated corn varieties. Because corn is essentially 100% cross pollinated, the corn varieties were a collection of heterozygous and heterogeneous individuals (genotypes). Varieties were developed by simple mass selection by the indigenous natives prior to the arrival of Columbus. Their methods of selection were simple by present-day standards, but they obviously were effective in developing races, varieties, and strains to satisfy their food, fuel, feed, and cultural needs. Hybridization occurred between varieties as cultures moved within the Western Hemisphere, releasing genetic variability to develop other unique varieties.

The fundamental concepts for development of hybrid corn were defined by 1920 (Sprague, 1946). Basic studies on the genetic composition of a corn variety were conducted to determine the effects of selfing (or inbreeding which is the opposite of outcrossing) within a corn variety (Shull, 1908). Because corn is naturally cross fertilizing, the genetic composition of each plant is not known. Continuous selfing of individuals for 7 to 10 generations resulted in pure lines (or inbred lines) within which every plant had similar traits. The correct interpretation of what occurred during inbreeding was based on Mendelian genetics: the heterozygous loci were eliminated by inbreeding to homozygous loci of either one of the two alleles at each locus. The fixation of alleles in pure lines caused a general reduction in vigor and productivity.

It was found upon crossing two pure lines that vigor was restored. If no selection occurred during inbreeding, the average performance (e.g., grain yield) of all possible crosses was similar to performance of the original variety in which inbreeding was initiated. Some crosses, however, were better than the original open-pollinated variety and could be reproduced from the cross of the pure-line parents of the cross. Hence, the concept of hybrid corn was determined (Shull, 1909): self to develop pure lines, cross the pure lines to produce hybrids, evaluate hybrids to determine the best hybrid, and use of pure-line parents to reproduce the superior hybrid and distribute it for use by the growers.

Hybridization is used in many phases of corn breeding because of the expression of heterosis. Hybridization is used to produce breeding populations (e.g., F_2) to develop inbred lines for use in hybrids, and hybridization is used to produce the crosses of superior lines for distribution to growers. Hybridization is easily accomplished either by hand pollinations or by wind pollination in large crossing fields (male and female inbred lines) to produce large quantities of high quality hybrid seed.

6. Potential for outcrossing

a. Outcrossing with wild Zea species:

Annual teosinte (2n = 20) and corn (2n = 20) are wind pollinated, tend to outcross, and are highly variable, interfertile species (Wilkes, 1972; 1989). Corn and teosinte are genetically compatible, and in areas of Mexico and Guatemala they freely hybridize when in proximity to each other. Teosinte exists primarily as a weed around the margins of the corn fields, and the frequency of hybrids between teosinte and corn has been studied. A frequency of one F_1 hybrid (corn x teosinte) for every 500 corn plants or 2 to 5% of the teosinte population for the Chalco region of the Valley of Mexico was reported (Wilkes, 1972). As stated, this frequency of hybrids represents a significant gene exchange between a wild weedy plant (*i.e.*, teosinte) and a cultivated relative (*i.e.*, corn) (Wilkes, 1972). The F_1 hybrid of teosinte by corn is robust and fertile and is capable of backcrossing to corn. Intercrossing and gene exchange between teosinte and corn occurs freely, and, accompanied by selection, teosinte had a significant role in the evolution of corn.

Corn easily crosses with teosinte, but teosinte is not present in the U.S. Corn Belt. The natural distribution of teosinte is limited to the seasonally dry, subtropical zone with summer rain along the western escarpment of Mexico and Guatemala and the Central Plateau of Mexico (Wilkes, 1972). Except for special plantings, teosinte is not found in the United States, and there have been no instances reported that teosinte occurs as a weed along the margins of corn plantings in the U.S. Corn Belt.

Tripsacum-corn hybrids have not been observed in the field and *Tripsacum*-teosinte hybrids have not been produced (Wilkes, 1972). *Tripsacum* evolved by polyploidy, whereas corn and teosinte have undergone introgressive hybridization at the diploid level (2n = 20). The diploid forms of *Tripsacum* (2n = 36) are morphologically distinct and allopathic in their distribution (Wilkes, 1989). *Tripsacum* species are perennials and seem to be more closely related to the genus *Manisuris* than to either corn or teosinte (Goodman, 1976). *Tripsacum* received greater interest in the evolution of corn after Mangelsdorf and Reeves (1931) successfully crossed corn and *Tripsacum dactyloides* (2n = 36). The cross by Mangelsdorf and Reeves (1931) was made with the diploid *Tripsacum dactyloides* (2n = 36) as the male parent. Silks of the female corn parent were cut to permit successful pollination. The cross had 28 chromosomes and was male sterile. Five other *Tripsacum* species have been crossed with corn, and has mapped more than 50 homologous loci on the

chromosomes of corn and *Tripsacum* (Galinat, 1988). In contrast with corn and teosinte being easily hybridized, both in the wild and by controlled pollinations, it requires special techniques to hybridize corn and *Tripsacum*. Except for *Tripsacum floridanum*, it is difficult to cross *Tripsacum* with corn, and the offspring of the cross show varying levels of sterility. Small portions of *Tripsacum* genome can be incorporated by backcrossing.

Sixteen species of *Tripsacum* have been described (Table II.1). *Tripsacum floridanum* is native to southern tip of Florida. Twelve of 16 *Tripsacum* are native to Mexico and Guatemala. *Tripsacum-australe* and two other species are native to South America. The center of variation for *Tripsacum* is the western slopes of Mexico, the same area where teosinte is frequently found. The habitat preferences of *Tripsacum* are similar to those for teosinte: seasonally dry, summer rains, elevation of 1500 m, and limestone soils (Wilkes, 1972).

b. Outcrossing with cultivated Zea varieties:

Corn is wind pollinated, and the distances that viable pollen can travel depend on prevailing wind patterns, humidity, and temperature. Occasionally it has been found that corn pollen can travel up to 3.2 km (2 miles) by wind under favorable conditions. All corns will interpollinate, except for certain popcorn varieties and hybrids that have one of the gametophyte factors (Ga^s, Ga, and ga allelic series on chromosome 4). Pollen of a specific hybrid can be carried by wind to pollinate other dent corn hybrids, sweet corn, and popcorn, if the popcorn does not carry the dent-sterile gametophyte factor. Corn pollen, therefore, moves freely within an area, lands on silks of the same cultivar or different cultivars, germinates almost immediately after pollination, and within 24 hours completes fertilization. Although there may be some minor differences in rate of pollen germination and pollen tube elongation on some genotypes, corn pollen is very promiscuous. It is estimated each corn plant can shed more than 10 million pollen grains.

Certification standards for distances between different corn genotypes have been established to assist in the production of hybrid corn having desired levels of purity. A specific isolation field to produce commercial hybrid seed shall be located so that the seed parent is no less than 200 m (640 feet or 40 rods) from other corn of a similar type; i.e., if seed parent is a yellow, dent corn it should be isolated at least 200 m from other yellow, dent corns. The distance of 200 m can be modified because of size of field, number of border rows, and different maturity dates of flower, provided no receptive silks are available at the time pollen is being shed from the contaminating field. If the hybrid seed being produced is of a different color or texture from neighboring contaminating fields, the distances and the number of border rows should be increased.

7. Weediness of corn

Modern-day corn cannot survive as a weed. One does not find volunteer corn growing in fence rows, ditches, and road sides as a weed. Although corn from the previous crop year can overwinter and germinate the following year, they

cannot persist as a weed. The appearance of corn in soybean fields following the corn crop from the previous year is a common occurrence. Measures are often taken to either eliminate the plants with the hoe or use of herbicides to kill the plants in soybean fields, but the plants that remain and produce seed usually do not persist the following years.

It is difficult for the corn to survive as a weed because of past selection in the evolution of corn. In contrast with weedy plants, corn has a polystichous female inflorescence (or ear) on a stiff central spike (or cob) enclosed with husks (modified leaves). Consequently, seed dispersal of individual kernels naturally does not occur because of the structure of the ears of corn. Individual kernels of corn, however, are distributed in fields and main avenues of travel from the field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities. In neither instance (natural or mechanical harvesting) does corn become a troublesome weed. Corn cannot survive without human assistance and is not capable of surviving as a weed.

B. Environmental Consequences of Introduction of the Transformed Variety

1. Weediness of a transformed corn variety

In the past 10 years, techniques have been developed for gene transfer into plants. Gene transformation is the acquisition by a cell of new gene(s) by the uptake of naked DNA, which can be by direct introduction of DNA and by either the Ti system or through protoplast transformations. One of the more common applications of gene transfer being used in corn is the introduction of gene(s) conferring insect resistance or tolerance to herbicides; i.e., insect resistance and herbicide tolerance. Herbicide tolerance is usually conferred by single genes that interact with key enzymes in important metabolic pathways. Insect resistance is typically conferred by expression of the *Bacillus thuringiensis* (*B.t.*) protein. The lines and hybrids that include the transferred gene(s) (e.g., herbicide tolerance and insect resistance) will have to meet the standards of nontransformed lines and hybrids to be competitive in the marketing of hybrid seed corn. The introduction of genes by the newer molecular techniques will be more precise than the classical backcross methods and will be directed primarily to single genes. The overall phenotype of transformed plants will be very similar to the original phenotype: the reproductive organs (tassels and ears), duration of plant development, methods of propagation, ability to survive as a weed, etc. will not change.

2. Potential for outcrossing of the transformed variety

a. Outcrossing with wild Zea species:

Outcrossing of transformed corn plants with wild relatives of corn will be the same as for nontransformed corn plants. Outcrossing with teosinte species will only occur where teosinte is present in Mexico and Guatemala.

Outcrossing with *Tripsacum* species is not known to occur in the wild and only under very carefully conditions can corn be crossed with *Tripsacum*. In the United States, only *Tripsacum floridanum* is known to be present in southern tip of Florida. Teosinte and *Tripsacum* are included in botanical gardens in the United States and the possibility exists, though unlikely, that the exchange of genes would occur between corn and its wild relatives. No cases of gene flow between corn and its wild relatives are known in the United States.

b. Outcrossing with cultivated Zea varieties:

Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time. Wind-blown pollen would move about among plants within the same field and among plants in nearby fields. Free flow of genes would occur similar to what occurs in cultivated corn. The transformed plants include individual genes and depending on the relative expression of the transformed genes (relative levels of dominance for gene expression), plant architecture, and reproductive capacities of the intercrossed plants will be similar to normal corn. The chances that a weedy type of corn will result from outcrossing with cultivated corn is extremely remote.

Table II.1. Taxonomic Classification of Corn and its Closely Related Relatives.

Family - Gramineae

Subfamily - Panicoideae

Tribe - Maydae

Western Hemisphere:

A. Genus - Zea
   I. Subgenus - Luxuriantes
      1. Zea luxurians (2n = 20)
      2. Zea perennis (2n = 40)
      3. Zea diploperennis (2n = 20)

   II. Subgenus - Zea
      1. Zea mays (2n = 20)
      Subspecies
      1. Zea parviglumis (2n = 20)
      2. Zea huehuetenangensis (2n = 20)
      3. Zea mexicana (Schrad.) (2n = 20)

B. Genus - Tripsacum
   Species --

andersomii (2n = 64)	latifolium (2n = 36)
australe (2n = 36)	percuvianum (2n = 72, 90, 108)
bravum (2n = 36, 72)	zopilotense (2n - 36, 72)
cundinamarce (2n = 36)	jalapense (2n = 72)
dactyloides (2n = 72)	lanceolatum (2n = 72)
floridanum (2n = 36)	laxum (2n = 36?)
intermedium (2n = 72)	maizar (2n = 36, 72)
manisuroides (2n = 72)	pilosum (2n = 72)

Asia:

Genera --
   Chionachne (2n = 20)
   Coix (2n = 10, 20)
   Polytoca (2n = 20)
   Schlerachne (2n = 20)
   Trilobachne (2n = 20)

Tribe -- Andropogoneae

A. Genus - Manisuris

C. References

Carlson, Wayne C. 1988. The cytogenetics of corn. p. 259-343. *In*: G. F. Sprague and J. W. Dudley (ed.) Corn and Corn Improvement. 3rd ed. American Society of Agronomy. Madison, WI.

Coe, E. H., Jr., M. G. Neulfer, and D. A. Hoisington. 1988. The genetics of corn. p. 81-258. *In*: G. F. Sprague and J. W. Dudley (ed.) Corn and Corn Improvement. 3rd ed. American Society of Agronomy. Madison, WI.

Doebley, J. F., and H. H. Iltis. 1980. Taxonomy of *Zea* (Gramineae). I. A subgeneric classification with key to taxa. Amer. J. Botany 67:982-993.

Galinat, Walton C. 1988. The origin of corn. p. 1-31. *In*: G. F. Sprague and J. W. Dudley (ed.) Corn and Corn Improvement. 3rd ed. American Society of Agronomy. Madison, WI.

Goodman, Major M. 1976. Maize. p. 128-136. In: N. W. Simmonds (ed.) Evolution of Crop Plants. Longman Group Ltd., London.

Goodman, Major M. 1988. The history and evolution of maize. CRC Critical Rev. Plant Sciences 7:197-220. CRC Press, Boca Raton, FL.

Goodman, M. M., and W. L. Brown. 1988. Races of corn. p. 33-79. *In*: G. F. Sprague and J. W. Dudley (ed.) Corn and Corn Improvement. 3rd ed. American Society of Agronomy, Madison, WI.

Hanway, John J. 1966. How a corn plant develops. Spec. Rep. No. 48. Iowa State Univ. Coop. Extension Service, Ames, IA.

Manglesdorf, Paul C. 1974. Corn - Its Origin, Evolution, and Improvement. Harvard Univ. Press. Cambridge, MA.

Manglesdorf, P.C., and R.C. Reeves. 1931. Hybridization of maize, *Tripsacum* and Euchlaena. J. Hered. 22:329-343.

Phillips, R. L., D. A. Somers, and K. A. Hibberd. 1988. Cell tissue culture and in vitro manipulation. p. 345-387. *In*: G. F. Sprague and J. W. Dudley (ed.) Corn and Corn Improvement. 3rd ed. American Society of Agronomy. Madison, WI.

Shull, G. H. 1908. The composition of a field of maize. Am. Breeders' Assoc. Rep. 4:296-301.

Shull, G. H. 1909. A pure line method of corn breeding. Am. Breeders' Assoc. Rep. 5:51-59.

Sprague, G. F. 1946. The experimental basis for hybrid maize. Biolog. Rev. 21:101-120.

Troyer, A. Forrest. 1994. Breeding early corn. p. 341-396. *In*: Arnel R. Hallauer (ed.) Specialty Corns. CRC Press, Inc. Boca Raton, FL.

Walbot, Virginia, and Joachin Messing. 1988. Molecular genetics of corn. p. 389-429. *In*: G. F. Sprague and J. W. Dudley (ed.) Corn and Corn Improvement. American Society of Agronomy. Madison, WI.

Wilkes, H. Garrison. 1972. Maize and its wild relatives. Science 177:1071-1077.

Wilkes, Garrison. 1989. Maize: domestication, racial evolution, and spread. p. 440-455. *In*: D. R. Harris and G. C. Hillman (ed.) Foraging and Farming - The Evolution of Plant Exploration. Unwin Hyman, London.

Part III. Description of the DNA Sequences and Transformation Methods Utilized in the Development of Insect-Protected Roundup Ready Corn Line MON 802

Introduction

Insect-protected Roundup Ready corn line MON 802 was generated using a particle acceleration transformation system with two plasmids vectors containing the following genes: the *cryIA(b),* CP4 EPSPS and *gox*. The *cryIA(b)* gene was inserted to confer resistance to certain lepidopteran insects while the CP4 EPSPS and *gox* genes produce proteins which confer glyphosate tolerance, a selective agent used to identify plant cells expressing the *cryIA(b)* gene and to confer glyphosate (Roundup) tolerance at the whole plant level. In addition to these three genes, a *nptII* gene which encodes the enzyme neomycin phosphotransferase II (NPTII) was introduced under the control of its own bacterial promoter, to enable plasmid manipulation in bacterial systems.

Molecular analysis was performed to characterize the integrated DNA present in the corn line. The genomic DNA was evaluated, using Southern blot analyses (Southern, 1975), for the number of sites into which the plasmid DNA integrated into the corn genome, and the integrity of the genes contained within the inserts.

A. The Donor Genes

A description of the DNA elements present on the plasmids used to produce this corn line is given in Table III.1. Each gene, *cryIA(b),* CP4 EPSPS and *gox*, is regulated by the enhanced CaMV 35S promoter and corn *hsp70* intron and contains the NOS 3' termination sequences.

1. *cryIA(b)* gene

The *cryIA(b)* gene used to produce corn line MON 802 is a modification of the *cryIA(b)* gene isolated from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 as designated (Höfte and Whitely, 1989). The native full length gene encoding the CryIA(b) protein and its complete nucleotide sequence were described (Fischhoff *et al.,* 1987).

The *cryIA(b)* gene is 3468 nucleotides in length and encodes a full-length *B.t.k.* HD-1 [CryIA(b)] protein of 1156 amino acids, which when subjected to trypsin yields an active trypsin-resistant protein product of approximately 600 amino acids *in planta* and *in vitro* (Lee *et al.,* 1995). The *cryIA(b)* gene sequence was modified to increase the levels of expression in corn using strategies similar to those as previously described (Perlak *et al.,* 1991). The gene encodes a CryIA(b) protein product identical to that found in nature (Fischhoff *et al.,* 1987). The deduced amino acid sequence for the CryIA(b) protein (Figure III.1) was reported previously to the USDA (Croon *et al.,* 1995).

Figure III.1 Deduced Amino Acid Sequence of the CryIA(b) Protein.

```
   1  MDNNPNINEC IPYNCLSNPE VEVLGGERIE TGYTPIDISL SLTQFLLSEF
  51  VPGAGFVLGL VDIIWGIFGP SQWDAFLVQI EQLINQRIEE FARNQAISRL
 101  EGLSNLYQIY AESFREWEAD PTNPALREEM RIQFNDMNSA LTTAIPLFAV
 151  QNYQVPLLSV YVQAANLHLS VLRDVSVFGQ RWGFDAATIN SRYNDLTRLI
 201  GNYTDHAVRW YNTGLERVWG PDSRDWIRYN QFRRELTLTV LDIVSLFPNY
 251  DSRTYPIRTV SQLTREIYTN PVLENFDGSF RGSAQGIEGS IRSPHLMDIL
 301  NSITIYTDAH RGEYYWSGHQ IMASPVGFSG PEFTFPLYGT MGNAAPQQRI
 351  VAQLGQGVYR TLSSTLYRRP FNIGINNQQL SVLDGTEFAY GTSSNLPSAV
 401  YRKSGTVDSL DEIPPQNNNV PPRQGFSHRL SHVSMFRSGF SNSSVSIIRA
 451  PMFSWIHRSA EFNNIIPSSQ ITQIPLTKST NLGSGTSVVK GPGFTGGDIL
 501  RRTSPGQIST LRVNITAPLS QRYRVRIRYA STTNLQFHTS IDGRPINQGN
 551  FSATMSSGSN LQSGSFRTVG FTTPFNFSNG SSVFTLSAHV FNSGNEVYID
 601  RIEFVPAEVT FEAEYDLERA QKAVNELFTS SNQIGLKTDV TDYHIDQVSN
 651  LVECLSDEFC LDEKKELSEK VKHAKRLSDE RNLLQDPNFR GINRQLDRGW
 701  RGSTDITIQG GDDVFKENYV TLLGTFDECY PTYLYQKIDE SKLKAYTRYQ
 751  LRGYIEDSQD LEIYLIRYNA KHETVNVPGT GSLWPLSAPS PIGKCAHHSH
 801  HFSLDIDVGC TDLNEDLGVW VIFKIKTQDG HERLGNLEFL EGRAPLVGEA
 851  LARVKRAEKK WRDKREKLEW ETNIVYKEAK ESVDALFVNS QYDRLQADTN
 901  IAMIHAADKR VHSIREAYLP ELSVIPGVNA AIFEELEGRI FTAFSLYDAR
 951  NVIKNGDFNN GLSCWNVKGH VDVEEQNNHR SVLVVPEWEA EVSQEVRVCP
1001  GRGYILRVTA YKEGYGEGCV TIHEIENNTD ELKFSNCVEE EVYPNNTVTC
1051  NDYTATQEEY EGTYTSRNRG YDGAYESNSS VPADYASAYE EKAYTDGRRD
1101  NPCESNRGYG DYTPLPAGYV TKELEYFPET DKVWIEIGET EGTFIVDSVE
1151  LLLMEE
```

2. CP4 EPSPS gene

The CP4 EPSPS gene was used as a plant selectable marker and to provide tolerance to glyphosate. The mode of action of glyphosate is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinruken and Amrhein, 1980). An EPSPS has been isolated from *Agrobacterium* sp. strain CP4 which has been shown to be highly resistant to glyphosate (Harrison *et al.,* 1993). The CP4 EPSPS protein represents one of many different EPSPSs found in nature (Schulz *et al.,* 1985), is highly tolerant to the inhibition by glyphosate and has high catalytic efficiency, compared to most EPSPSs (Barry *et al.,* 1992; Padgette *et al.,* 1991). When plants are treated with glyphosate, the plants or plant cells expressing the CP4 EPSPS protein are unaffected since the continued action of the tolerant EPSPS enzyme meets the plant's need for aromatic compounds. Corn plants tolerant to glyphosate were produced by stably inserting both the CP4 EPSPS and *gox* genes into the chromosome of corn.

The CP4 EPSPS gene from *Agrobacterium* sp. strain CP4, has been completely sequenced and encodes a 47.6 kD protein consisting of a single polypeptide of 455 amino acids. The EPSPS from *Agrobacterium* sp. strain CP4 is naturally highly tolerant to inhibition by glyphosate and has high catalytic activity, compared to most glyphosate tolerant EPSPSs (Barry *et al.,* 1992; Padgette *et al.,* 1991). The bacterial isolate, CP4, was identified by the American Type Culture Collection as an *Agrobacterium* species. There is no human or animal pathogenicity known from *Agrobacterium* species, nor is the EPSPS gene a determinant of *Agrobacterium* plant pathogenesis.

The CP4 EPSPS gene contains a chloroplast transit peptide, CTP2, isolated from *Arabidopsis thaliana* EPSPS (Klee *et al.,* 1987) which directs the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid synthesis (Kishore and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (della-Cioppa *et al.,* 1986). The CP4 EPSPS gene with its CTP2 is approximately 1.7 Kb in size. The amino acid sequence of CP4 EPSPS with its CTP is given in Figure III.2 and was reported previously to the USDA (Croon *et al.,* 1995).

Figure III.2 Deduced Amino Acid Sequence of the CP4 EPSPS Protein. Sequence includes the CTP2 transit peptide (amino acids 1-76 are the transit peptide).

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

3. *gox* gene

The *gox* gene, cloned from *Achromobacter* sp. strain LBAA, was also inserted to provide tolerance to glyphosate. The *gox* gene encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX) (Hallas *et al.,* 1988; Barry *et al.,* 1992; Barry *et al.,* 1994). The GOX enzyme degrades glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate. The *gox* gene encodes a 46.1 kD protein. The GOX protein is targeted to the plastids with a chloroplast transit peptide sequence, CTP1. CTP1 was derived from the small subunit gene of ribulose-1,5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* (Timko *et al.,* 1988). The amino acid sequence of GOX with its CTP is given in Figure III.3 and was reported previously to the USDA (Croon *et al.,* 1995).

Figure III.3 Deduced Amino Acid Sequence of the GOX Protein including the CTP1 transit peptide (amino acids 1-88 are the transit peptide).

```
  1  MASSMLSSAT MVASPAQATM VAPFNGLKSS AAFPATRKAN NDITSITSNG
 51  GRVNCMQVWP PIGKKKFETL SYLPDLTDSG GRVNCMQAMA ENHKKVGIAG
101  AGIVGVCTAL MLQRRGFKVT LIDPNPPGEG ASFGNAGCFN GSSVVPMSMP
151  GNLTSVPKWL LDPMGPLSIR FSYFPTIMPW LIRFLLAGRP NKVKEQAKAL
201  RNLIKSTVPL IKSLAEEADA SHLIRHEGHL TVYRGEADFA KDRGGWELRR
251  LNGVRTQILS ADALRDFDPN LSHAFTKGIL IEENGHTINP QGLVTLLFRR
301  FIANGGEFVS ARVIGFETEG RALKGITTTN GVLAVDAAVV AAGAHSKSLA
351  NSLGDDIPLD TERGYHIVIA NPEAAPRIPT TDASGKFIAT PMEMGLRVAG
401  TVEFAGLTAA PNWKRAHVLY THARKLLPAL APASSEERYS KWMGFRPSIP
451  DSLPVIGRAT RTPDVIYAFG HGHLGMTGAP MTATLVSELL AGEKTSIDIS
501  PFAPNRFGIG KSKQTGPAS
```

4. *nptII* bacterial gene

The *nptII* gene which codes for the enzyme neomycin phosphotransferase II (NPTII) is also on the plasmids. This enzyme confers resistance to aminoglycoside antibiotics (*i.e.*, kanamycin and neomycin) and is necessary for the selection of bacteria during the construction of this plasmid. The coding sequence for the *nptII* gene was derived from the prokaryotic transposon Tn5 (Beck *et al.*, 1982) and is present under its own bacterial promoter. The promoter for this gene is only active in bacterial cells. The deduced amino acid sequence of the NPTII protein was reported previously to the USDA (Croon *et al.*, 1995).

5. Plasmid backbone sequences

The *alpha* region of the *lacZ* gene for beta-galactosidase, is present under a bacterial promoter. This region contained a polylinker (region with multiple cloning sites) which allowed for the cloning of the desired genes within the plasmid vector (Vieira and Messing, 1987). The *lacZ-alpha* region is followed by the 0.7 Kb origin of replication for the pUC plasmids (*ori-p*UC) which allows for the replication of plasmids in *E. coli* (Vieira and Messing, 1987).

B. Particle Acceleration Transformation System

Plasmid DNA was introduced into the plant tissue by the particle acceleration method (Klein *et al.*, 1987). DNA is precipitated onto microscopic tungsten or gold particles using calcium chloride and spermidine. A drop of the coated

particles is then placed onto a plastic macrocarrier, which is accelerated at a high velocity through a barrel by the explosive force of a gunpowder discharge. The macrocarrier hits a plastic stopping plate which stops the flight of the macrocarrier but allows continued flight of the DNA-coated particles. The particles penetrate the target plant cells, where the DNA is deposited and incorporated into the cell chromosome. The cells are incubated on a tissue culture medium containing 2,4-D which supports callus growth. The introduced DNA contains genes encoding for herbicide tolerance (e.g., the CP4 EPSPS and *gox* genes conferring tolerance to glyphosate). The plant cells are grown in the presence of glyphosate and only the transformed cells continue to grow. Plants are regenerated from the tolerant callus tissue, and assayed for the presence of the expressed CryIA(b) protein product and glyphosate tolerance.

C. The Recipient Corn Material

The corn plant tissue that is the recipient of the introduced plasmid DNA is a High Type II (Hi-II) genetic material. Hi-II is a deriviative of the A188 and B73 inbred lines of corn. These are publicly-available inbred lines developed in the U.S. by the University of Minnesota and Iowa State University, respectively (Armstrong *et al.,* 1991) and have been extensively used by corn breeders to produce new varieties for the U.S. and internationally. The material was developed to have a higher regeneration potential during the tissue culture stages along with acceptable commercial performance in hybrids.

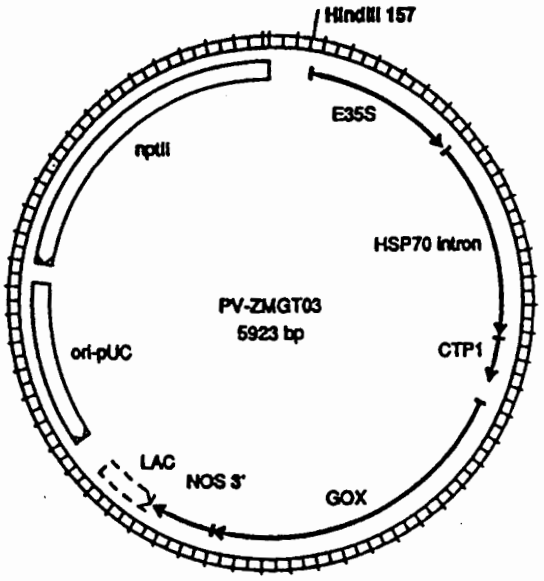
D. Plasmid Vectors Utilized for Transformation

Insect-protected Roundup Ready corn line MON 802 was produced using vectors PV-ZMGT03 and PV-ZMBK15 (Figure III.4). Plasmid vector PV-ZMGT03 contains *gox* gene while PV-ZMBK15 contains the *cryIA(b)* and CP4 EPSPS genes. In addition, each of the plasmids contains the *nptII* selectable marker gene, an origin of replication necessary for replicating the respective plasmid in *E. coli* and the *lacZ* region which includes a multi-linker cloning site (Table III.1). The corn genotype Hi-II was transformed (Armstrong *et al.,* 1991) with the plasmid vectors and particle acceleration method identified above.

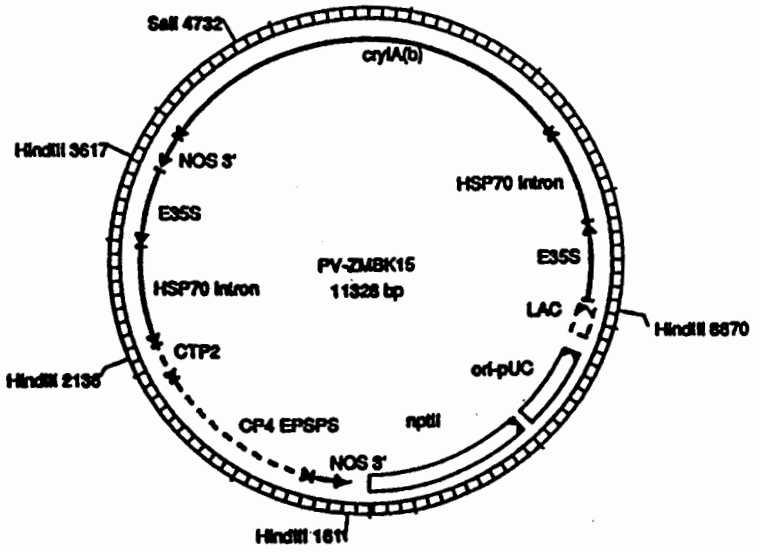
Table III.1 Summary of DNA Components of the Plasmids

Genetic Element	Size Kb	Function
E35S	0.64	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1985).
hsp70	0.81	Intron from the corn *hsp70* gene (heat-shock protein) present to increase the levels of gene transcription (Rochester *et al.*, 1986).
cryIA(b)	3.47	The gene encodes a full-length *Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) protein product identical to that found in nature (Fischhoff *et al.*, 1987).
CTP2	0.31	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS (Klee *et al.*, 1987), present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis.
CP4 EPSPS	1.4	The gene for CP4 EPSPS, isolated from *Agrobacterium* sp. strain CP4 (Harrison, *et al.*, 1993) allows for the selection of transformed cells on glyphosate and provides tolerance to glyphosate (Roundup) at the whole plant level.
NOS3'	0.27	A 3' nontranslated region of the nopaline synthase gene which terminates transcription and directs polyadenylation (Fraley, *et al.*, 1983). This sequence was derived from the Ti plasmid of *Agrobacterium.*
CTP1	0.26	Chloroplast transit peptide, isolated from the small subunit gene of ribulose-1,5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* (Timko *et al.*, 1988), present to direct the GOX protein to the chloroplast, the site of aromatic amino acid synthesis.
gox	1.3	The gene encodes the glyphosate metabolizing enzyme, glyphosate oxidoreductase (GOX), isolated from *Achromobacter* sp. (new genus *Ochrobactrum anthropi*) strain LBAA (Hallas *et al.*, 1988; Barry *et al*, 1992; Barry *et al.*, 1994). GOX allows for both the selection of transformed cells on glyphosate and tolerance to glyphosate at the whole plant level.
lacZ	0.62	A partial *E. coli lacI* coding sequences, the promoter Plac, and a partial coding sequence for beta-D-galactosidase or *lacZ* protein from pUC119 (Yanich-Perron *et al.*, 1985).
ori-pUC	0.65	The origin of replication for the pUC plasmids that allows for plasmid replication in *E. coli* (Vieira and Messing, 1987).
nptII	1.14	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). The coding sequence for the *nptII* gene was derived from the prokaryotic transposon Tn5 (Beck *et al.*, 1982) and is present under its own bacterial promoter.

Figure III.4 Plasmid maps of PV-ZMGT03 and PV-ZMBK15



Plasmid map of PV-ZMGT03, one of the vectors used to produce MON 802, including restriction sites used in Southern analysis.



Plasmid map of PV-ZMBK15, one of the vectors used to produce MON 802, including restriction sites used in Southern analysis.

E. Genetic Analysis of Insect-Protected Roundup Ready Corn Line MON 802

1. Summary

Corn line MON 802 was produced by particle acceleration technology with the two plasmids PV-ZMBK15 and PV-ZMGT03 that contain the *cryIA(b)*, CP4 EPSPS and *gox* genes, and the *nptII*/*ori-p*UC backbone. The maps of the two plasmids are presented in Figure III.4, along with the locations of various restriction sites. MON 818 control DNA and MON 802 DNA were digested with NdeI, the DNA fragments separated by gel electrophoresis and transferred to membranes which were probed with the whole plasmids, the *cryIA(b)* gene, the CP4 EPSPS gene, the *gox* gene, *nptII* and *ori-p*UC. Southern blot analyses established that corn line MON 802 contains two inserts (Table III.2). One insert, approximately 23 Kb, contains the *cryIA(b)*, CP4 EPSPS and *gox* genes and the *nptII*/*ori-p*UC backbone. The second insert, approximately 8 Kb, contains only the *gox* gene and the *nptII*/*ori-p*UC backbone. The Southern blots (Figures III.5 and III.6) contain results for an additional corn line, MON 805, which is not the subject of this application, and therefore, will not be discussed.

Table III.2 Summary of the Genetic Analysis of Insect-Protected Roundup Ready Corn Line MON 802

Genetic Element	23 Kb insert	8 Kb insert
cryIA(b) gene	present	absent
CP4 EPSPS gene	present	absent
gox gene	present	present
nptII/*ori-p*UC	present	present

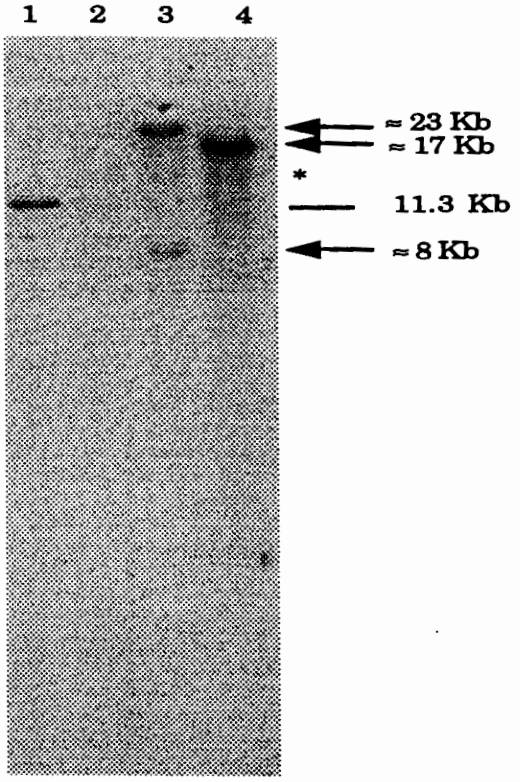
2. Insert number

The purpose of the NdeI digest was to enable the determination of the number of plasmid DNA inserts in the corn line MON 802. The plasmids PV-ZMBK15 and PV-ZMGT03 do not contain a restriction site for NdeI. Thus this enzyme effectively cuts the genomic DNA outside any inserted DNA, releasing a fragment(s) containing the inserted DNA. Figure III.5 shows the Southern blot of the NdeI digestions hybridized with a combination of PV-ZMBK15 and PV-ZMGT03 plasmid probes. Lane 1 contains PV-ZMBK15 digested with SalI, an enzyme that cuts once within the plasmid. The expected size band of 11.3 Kb was detected, as predicted by the plasmid map (Figure III.4). MON 818 control DNA, lane 2, was digested with NdeI and produced a barely detectable band of less than 17 Kb which is also detected in lanes 3 and 4. These bands are non-specific binding and are considered background. MON 802 DNA, lane 3, digested with NdeI, produced two bands: approximately 23 Kb and 8 Kb. These results established that plasmid DNA inserted at two loci.

3. Genetic elements present on each insert

Figure III.6 shows five Southern blots containing genomic MON 802 DNA digested with NdeI and probed with the following genetic elements: *nptII, cryIA(b), ori-p*UC, *gox* and CP4 EPSPS. In Panels A, B, C, and E, PV-ZMBK15 plasmid DNA, lane 1, was digested with SalI while in Panel D PV-ZMGT03 plasmid DNA, lane 1, was digested with HindIII. The bands shown for all plasmid DNA (all lane 1) are the predicted size, depending on the probe used, based on the plasmid maps (Figure III.4). MON 818 control DNA, lane 2 on all blots, was digested with NdeI. No bands were detected in lane 2 on any of the blots except for Panel C, probed with *ori-*pUC. The band was considered a background band since it appears in all lanes containing genomic DNA. MON 802 DNA, lane 3 on all blots, contained a 23 Kb band which was detected with each probe: *nptII* (Panel A), *cryIA(b)* (Panel B), *ori-*pUC (Panel C), *gox* (Panel D), and CP4 EPSPS (Panel E). The 8 Kb band hybridized to *nptII, ori-p*UC and *gox* probes. This data established that the 23 Kb insert in MON 802 contains the following elements: *cryIA(b)*, *gox*, and CP4 EPSPS genes, and the *nptII / ori-p*UC backbone. The 8 Kb insert contains only the *gox* gene and the *nptII / ori-pUC* backbone. Corn line MON 802 contains two inserts which contain the elements listed in Table III.2.

Figure III.5 MON 802 and MON 805: Insert Number Analysis



PV-ZMBK15 and
PV-ZMGT03 probes

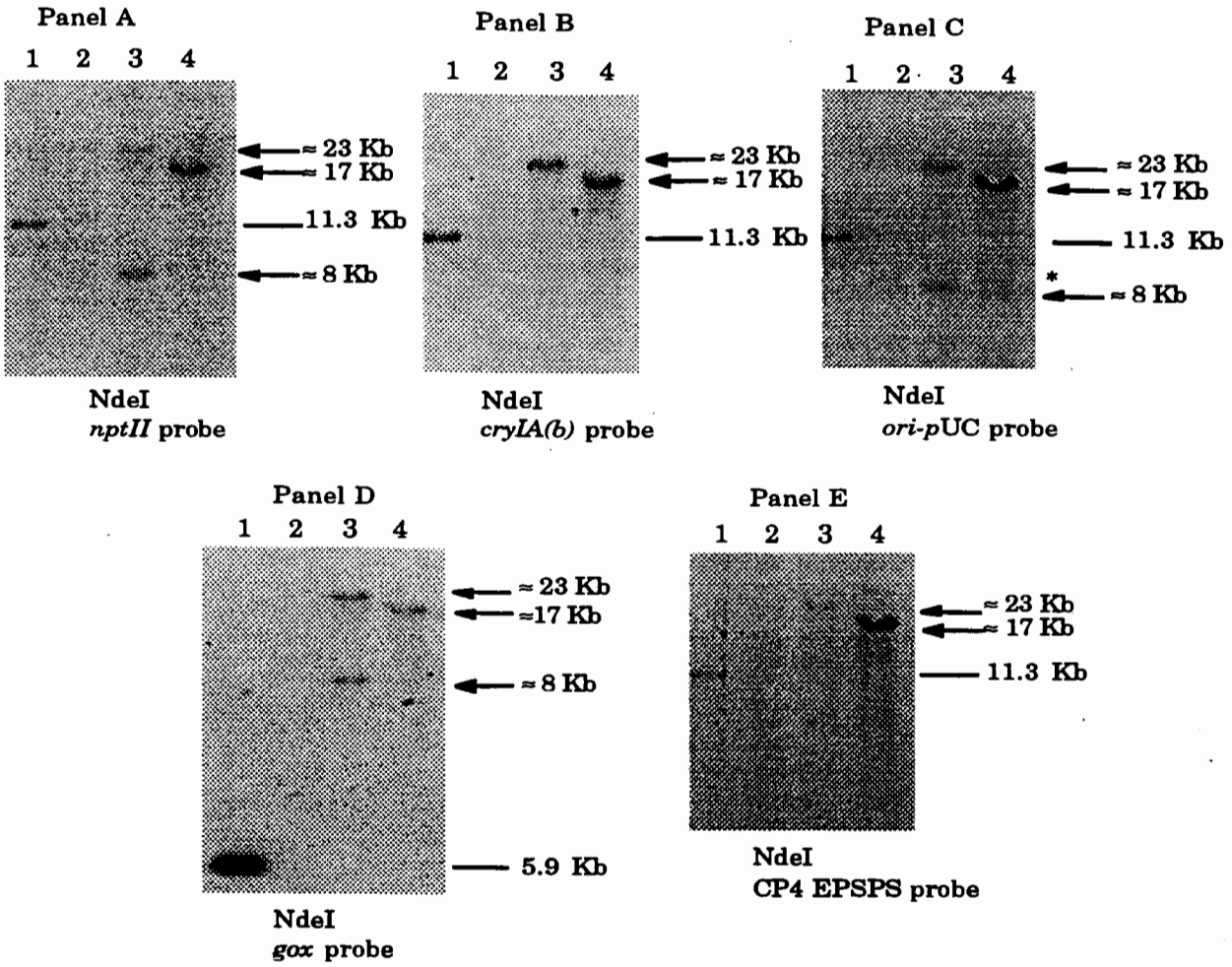
Southern blot analysis of maize line MON 802 and MON 805 DNA: insert number analysis. Lane 1 contains 100 pg of plasmid PV-ZMBK15 DNA digested with SalI. Lanes 2-4 contain 5 µg of the following DNAs digested with NdeI and probed with a combination of plasmids PV-ZMBK15 and PV-ZMGT03: lane 2, MON 818 DNA; lane 3, MON 802 DNA; lane 4, MON 805 DNA.

——— Symbol denotes sizes obtained from plasmid digests.

—►≈ Symbol denotes a band size approximated from MW markers and plasmid digests.

* Symbol denotes background bands.

Figure III.6 MON 802 and MON 805: Genetic Element Analysis



Southern blots analysis of maize lines MON 802 and MON 805 DNA: *nptII*, cryIA(b), *ori*-pUC, gox, and CP4 EPSPS gene analysis.
Lane 1 of panels A-C and E contained 100 pg of plasmid PV-ZMBK15 digested with SalI. Lane 1 of panel D contained 100 pg of plasmid PV-ZMGT03 digested with HindIII. Lanes 1-4 contained 5 µg of the following DNAs digested with NdeI: lane 2, MON 818 DNA; lane 3, MON 802 DNA; lane 4, MON 805 DNA. The membranes were probed with the following ^{32}P labelled elements: panel A, *nptII* gene; panel B, *cryIA(b)* gene; panel C, *ori*-pUC; panel D, *gox* gene; panel E, CP4 EPSPS gene.

——— Symbol denotes sizes obtained from plasmid digests.
—→≈ Symbol denotes a band size approximated from MW markers and plasmid digests.
* Symbol denotes background bands.

F. Segregation Data and Stability of Gene Transfer

1. Insect-protected Roundup Ready corn line MON 802

Segregation data for three generations of MON 802 are presented in Table III.3. Data is presented for the BC0 F1 plants (derived from crossing the R0 with an inbred line), BC1 F1 plants (derived from crossing the BC0 F1 plants to the same inbred used to cross with the R0 plant), and BC1 F2 progeny (derived from crossing individual BC1 F2 plants by a non-transgenic tester and analyzing the subsequent generation ear to row).

Statistical significance for the segregation data was determined using Chi Square analyses. For these analyses, a Chi Square value (X^2) was determined as follows: $X^2 = \sum [(o-c)^2/c]$ where o= observed frequencies and c= calculated frequencies for the various classes. Calculated and observed frequencies were determined as described in footnotes of the table. The calculated value was compared to a Table of Chi Square to determine whether the observed frequencies fit the expectation for a single gene insert at p=0.05 and/or p=0.01.

The Chi square analyses of the segregation results for MON 802 are consistent for a single locus segregating according to Mendelian genetics (Table III.3).

Table III.3 Segregation Data and Analysis of Progeny of Insect-Protected Roundup Ready Corn Line MON 802

Generation	Actual	Expected	ChiSq
BC0F1[1]	10:10	10:10	0.00*
BC1F1[2]	101:108	104.5:104.5	0.23*
BC1F2 progeny[3]	258:480:198	234:468:234	8.31#

[1] Data expressed as number of expressing plants: number of non-expressing plants based on European corn borer (ECB) feeding assay.
[2] Data expressed as number of expressing plants: number of non-expressing plants based on ECB feeding assay or glyphosate spray.
[3] Data expressed as number of ear rows with homozygous expressing plants: number of ear rows with segregating plants: number of ear rows with homozygous susceptible plant based on glyphosate sprays.
*not significant at p = 0.05 (chi square = 3.84, 1 df).
#significant at p = 0.05 (chi square = 5.99, 2 df); significant at p = 0.01 (chi square = 9.21, 2 df).

The inserts in insect-protected Roundup Ready corn line MON 802 have been shown to be stable through six generations of crosses to one recurrent parent (B73) and four generations of crosses to a second unrelated inbred (Mo17) (Table III.4).

Table III.4 Segregation Data for Backcross Derivatives of Insect-Protected Roundup Ready Corn Line MON 802 in Two Unrelated Inbred Lines (B73 and Mo17)

Generation	Actual	Expected	ChiSq
BC5F1(B73)[1]	39:29	34:34	1.47*
BC3F1(Mo17)[2]	41:23	32:32	5.06#

[1] Data expressed as number of expressing plants: number of non-expressing plants based on CryIA(b) ELISA.
[2] Data expressed as number of homozygous progeny: number of segregating or homozygous non-expressing progeny *cryIA(b)* gene by PCR.
* not significant at p = 0.05 (chi square = 3.84, 1 df).
# not significant at p = 0.01 (chi square = 6.64, 1 df).

To summarize the segregation and stability data (Tables III.3 and III.4), the data are consistent with co-segregation of the two closely-linked inserts containing the *cryIA(b)*, CP4 EPSPS and *gox* genes in the genomic DNA of line MON 802. The stability of these inserts has been demonstrated through six generations of crossing.

G. Conclusion

Insect-protected Roundup Ready corn line MON 802 was produced by particle acceleration technology with plasmids containing *cryIA(b)*, CP4 EPSPS and *gox* genes. Corn line MON 802 contains two closely linked inserts. The ≈23 Kb insert contains the *cryIA(b)*, CP4 EPSPS and *gox* genes and the *nptII*/*ori-p*UC backbone. The ≈8 Kb insert contains the *gox* gene and the *nptII*/*ori-p*UC backbone.

H. References

Armstrong, C.L., C.E. Green, and R.L. Phillips. 1991. Development and availability of germplasm with high Type II culture formation response. Maize Genetics Cooperation Newsletter 65:92-93.

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

Barry, G.F., M.L. Taylor, S.R. Padgette, K.H. Kolacz, L.E. Hallas, G. della-Cioppa, and G.M. Kishore. 1994. Cloning and Expression in *Escherichia coli* of the Glyphosate-to-Aminomethylphosphonic Acid Degrading Activity from *Achromobacter* sp. strain LBAA. Monsanto Technical Report MSL-13245, St. Louis.

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss, and H. Schaller. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. Gene 19:327-336.

Croon, K.A., P.R. Sanders, P.J. Keck, T.C. Lee, and G.B. Parker. 1995. Petition for Determination of Nonregulated Status: Insect-protected Corn (*Zea mays* L.) with the cryIA(b) Gene from *Bacillus thuringiensis* subsp. *kurstaki*. USDA Petition no. 95-093-01P.

della-Cioppa, G., C.S. Bauer, B.K. Klein, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1986. Translocation of the Precursor of 5-Enolpyruvylshikimate-3-phosphate Synthase into Chloroplasts of Higher Plants *in vitro*. Proc. Natl., Acad. Sci. USA 83:6873-6877.

Fischhoff, D.A., K.S. Bowdish, F.J. Perlak, P.G. Marrone, S.M. McCormick, J.G. Niedermeyer, D.A. Dean, K. Kusano-Kretzmer, E.J. Mayer, D.E. Rochester, S.G. Rogers, and R.T. Fraley. 1987. Insect tolerant transgenic tomato plants, Bio/technology 5:807-813.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann, and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proc. Natl. Acad. Sci. USA, 80:4803-4807

Hallas, L.E., E.M. Hahn, and C. Korndorfer. 1988. Characterization of microbial traits associated with glyphosate biodegradation in industrial activated sludge. J. Industrial Microbiol. 3:377-385.

Harrison, L.A., M.R. Bailey, R.M. Leimgruber, C.E. Smith, D.L. Nida, M.L. Taylor, M. Gustafson, B. Heeren, and S.R. Padgette. 1993. Characterization of Microbially-Expressed Protein: CP4 EPSPS. Monsanto Technical Report MSL-12901, St. Louis.

Höfte, H. and H.R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiological Reviews 53:242-255.

Kay, R., A. Chan, M. Daly, and J. McPherson. 1985. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. Science 236: 1299-1302.

Kishore, G. and D. Shah. 1988. Amino acid biosynthesis inhibitors as herbicides. Ann, Rev. Biochem. 57:627-663.

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

Klein, T.M., E.D. Wolf, R. Wu, and J.C. Sanford. 1987. High velocity microprojectiles for delivering nucleic acids into living cells. Nature 327:70-73.

Lee, T.C., M. Bailey, and P.R. Sanders. 1995. Compositional Comparison of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein Produced in ECB Resistant Corn and the Commercial Microbial Product, DIPEL. An unpublished study conducted by Monsanto Company. EPA MRID 43533203.

Odell, J.T., F. Nagy, and N-H Chua. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313: 810-812.

Padgette, S.R., D.B. Re, C.S. Gasser, D.A. Eichholtz, R.B. Frazier, C.M. Hironaka, E.B. Levine, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1991. Site-directed mutagenesis of a conserved region of the 5-enolpyruvylshikimate-3-phosphate synthase active site. J. Biol. Chem. 266:22364-22369.

Perlak, F.J., Fuchs, R.L., Dean, D.A., McPherson, S.L., Fishhoff, D.A. 1991. Modification of the coding sequence enhances plant expression of insect control protein genes. Proc. Natl. Acad. Sci. USA 88:3324-3328.

Rochester, D.E., J.A. Winer, and D.M. Shah. 1986. The structure and expression of maize genes encoding the major heat shock protein, hsp70. EMBO J. 5:451-458.

Schulz, A. , A. Krüper, and N. Amrhein. 1985. Differential sensitivity of bacterial 5-enolpyruvylshikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiology Letters 28:297-301.

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

Steinrüken, H.C. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimate-3-phosphate synthase. Biochem. Biophys. Res. Comm. 94:1207-1212.

Timko, M.P., Herdies, L., de Almeida, E., Cashmore, A.R., Leemans, J., and Krebbers, E. 1988. Genetic engineering of nuclear-encoded components of the photosynthetic apparatus in *Arabidopsis*. pp. 279-295. *In* "The Impact of Chemistry on Biotechnology," ACS Books.

Vieira, J. and J. Messing. 1987. Production of single-stranded plasmid DNA. Methods Enzymol. 153:3-11.

Yanich-Perron, C., J. Vieira and J. Messing. 1985. Improved M13 Phage Cloning Vectors and Host Strains: Nucleotide Sequences of the M13 mp18 and pUC19 Vectors. Gene 33:103-119.

Part IV. Detailed Description of the Phenotype of Insect-protected Roundup Ready Corn Line MON 802

Introduction

Data and information supplied in this Petition for Determination of Non-Regulated Status demonstrate that insect-protected Roundup Ready corn line MON 802 is substantially equivalent to non-modified corn, except for the inserted genetic sequences, the expressed proteins [CryIA(b), CP4 EPSPS and GOX proteins], the ability of the plant to resist damage from certain lepidopteran insects and tolerance to the herbicide Roundup. The information supplied in this section and referenced from other sections of this petition will demonstrate that the modified corn line is not likely to pose a greater plant pest risk than non-modified corn. This conclusion is based on evaluation of phenotypic characteristics, safety of the expressed proteins, and the lack of any deleterious environmental fate/effects.

A variety of studies were conducted to characterize the unique traits of the modified corn line and to establish that this insect-protected Roundup Ready corn line is substantially equivalent to non-modified corn. These include:

- expression of the CryIA(b), CP4 EPSPS and GOX proteins
- safety assessment of the CryIA(b) protein to non-target insects
- the environmental fate of the CryIA(b) protein
- the potential for outcrossing and weediness
- field germination results
- disease and pest susceptibility
- the comparison of line MON 802 and parental controls, on the basis of composition of the corn seed

The following sections summarize these investigations.

A. The CryIA(b) Protein

The CryIA(b) protein must be ingested by the insect to exert insecticidal activity (Huber and Lüthy, 1981). The protein in its crystalline form is insoluble in aqueous solution at neutral or acidic pH (Bulla *et al.*, 1977); however, the pH of the larval insect gut is alkaline which favors solubilization of the protein crystal. The solubilized protein is subsequently activated by proteases in the insect gut. The activated protein, which consists of approximately 600 amino acids, diffuses through the peritrophic membrane of the insect to the midgut epithelium, binding to the specific high affinity receptors on the surface of the midgut epithelium of target insects (Wolfersberger *et al.*, 1986; Hofmann *et al.*, 1988a). The gut becomes paralyzed as a consequence of changes in electrolytes and pH in the gut causing the larval insect to quit feeding and die.

There are no receptors for the protein delta-endotoxins of *Bacillus thuringiensis* subspecies on the surface of mammalian intestinal cells, therefore, humans are not susceptible to these proteins (Hofmann *et al.*, 1988b; Noteborn, 1994; Sacchi *et al.*, 1986). In addition to the lack of receptors for the *B.t.k.* proteins, the absence of adverse effects in humans is further supported by numerous reviews on the safety of the *B.t.* protein (Ignoffo, 1973; Shadduck, 1983; Siegel and Shadduck, 1989) and by our rodent feeding (Naylor, 1992) and *in vitro* digestive fate studies of the *B.t.k.* CryIA(b) protein (Ream, 1994).

Data was submitted to the EPA to support the registration and exemption from the requirement of a tolerance for the CryIA(b) protein as a plant pesticide. Studies included within that submission demonstrate the safety of this protein. In a mouse acute oral gavage study, no treatment related effects were observed in any of the groups of mice administered the CryIA(b) trypsin-resistant core protein by oral gavages at dosages up to 4000 mg/kg. The oral LD_{50} for the CryIA(b) protein in mice is greater than 4000 mg/kg and the no effect level is 4000 mg/kg (Naylor, 1992).

In an *in vitro* mammalian digestion study, the CryIA(b) protein degraded rapidly; more than 90% of the initially added CryIA(b) protein degraded after two minutes incubation in simulated gastric fluid as detected by western blot analysis and insect bioassay. In intestinal fluid, the trypsin-resistant core of the CryIA(b) protein did not degrade substantially after approximately 19.5 hours incubation as assessed by both western blot analysis and by insect bioassay (Ream, 1994). This result was expected as the trypsin-resistant core of this and other *B.t.* insecticidal proteins have been shown to be relatively resistant to digestion by trypsin (Bielot *et al*, 1989). These results are fully consistent with the history of safe use of *B.t.* preparations by humans.

B. The CP4 EPSPS Protein

EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants (including corn) and microorganisms (Levin and Sprinson, 1964; Steinrüken and Amrhein, 1980), and is thus ordinarily present in food derived from plant sources. Genes for numerous EPSPS's have been cloned (Padgette *et al.*, 1989, 1991), and active site domains are conserved among the known EPSPSs (Padgette *et al.*, 1988, 1991). Bacterial EPSPSs have been well-characterized with respect to the 3-dimensional X-ray crystal structure (Stallings *et al.*, 1991) and the detailed kinetic and chemical reaction mechanism (Anderson and Johnson, 1990). EPSPSs from a number of bacteria exhibit tolerance to glyphosate (Schulz *et al.*, 1985). CP4 EPSPS thus represents one of many different EPSPSs found in nature.

The herbicide glyphosate kills plants cells in the transformation process due to inhibition of the enzyme EPSPS (Steinrücken and Amrhein, 1980). The aromatic amino acid pathway is not present in mammalian metabolic pathways (Cole, 1985). This contributes to the selective action of glyphosate toward plants but not mammals. Glyphosate tolerance can be conferred to plant cells and microbes by either overproduction of EPSPS or the use of glyphosate-tolerant EPSPSs. The EPSPS from *Agrobacterium* sp. strain CP4 is highly tolerant to inhibition by glyphosate and has high catalytic efficiency, compared to most glyphosate-tolerant EPSPSs (Barry *et al.*, 1992; Padgette *et al.*, 1991). Upon glyphosate treatment, the corn cells in the transformation process expressing the CP4 EPSPS are unaffected since the continued action of the glyphosate-tolerant EPSPS enzyme meets the plant's need for aromatic compounds.

CP4 EPSPS is a 47.6 KD protein consisting of a single polypeptide of 455 amino acids. The gene encoding CP4 EPSPS has been completely sequenced. The enzyme has been expressed in *E. coli* and highly purified. CP4 EPSPS interacts with the EPSPS substrates shikimate-3-phosphate and phosphoenolpyruvate similarly to the plant enzymes, based on steady-state kinetic analyses. In addition, recent results indicate that the 3-dimensional X-ray crystal structure of CP4 EPSPS exhibits the same overall folding pattern as the *E. coli* EPSPS enzyme.

The isolate CP4 was identified by the ATCC (American Type Culture Collection) as an *Agrobacterium* species, hence the designation *Agrobacterium* sp. strain CP4. Agrobacteria occur almost worldwide in soils and in the rhizosphere of plants. *Agrobacterium* strains have also been reported in a number of human clinical specimens, but it is believed that these clinical *Agrobacterium* isolates occur either as incidental inhabitants in the patient or as contaminants introduced during sample manipulation (Kersters and De Ley, 1984).

The chloroplast transit peptide (CTP) coding sequence from petunia EPSPS (Shah *et al.*, 1986; Gasser *et al.*, 1988) has been fused to the 5'-end of the CP4 EPSPS gene to deliver the CP4 EPSPS to the chloroplasts, the site of EPSPS activity and glyphosate action. Plant expression of the gene fusion produces a pre-protein which is rapidly imported into chloroplasts where the CTP is cleaved and degraded, releasing the mature CP4 EPSPS protein (della-Cioppa *et al.*, 1986).

C. The GOX Protein

The *gox* gene, cloned from *Achromobacter* sp. strain LBAA (new genus *Ochrobactrum anthropi*), was also inserted to provide tolerance to glyphosate. *Achromobacter* species are reported to be one of the most frequently occurring bacteria in the rhizosphere (Joos *et al.*, 1988) and the enzyme has been extensively characterized (Padgette *et al.*, 1994). The *gox* gene encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX) (Hallas *et al.*, 1988; Barry *et al.*, 1992; Barry *et al.*, 1994). The GOX enzyme degrades glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate. The *gox* gene encodes a 46.1 kD protein. The GOX protein is targeted to the plastids with a chloroplast transit peptide sequence, CTP1. CTP1 was derived from the small subunit gene of ribulose-1, 5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* (Timko et al., 1988). The amino acid sequence of GOX with its CTP is given in Figure III.3 and was reported previously (Croon et al., 1995).

D. Expression Levels of the CryIA(b), CP4 EPSPS and GOX Proteins in Corn Line MON 802

Levels of the expressed proteins were evaluated in young leaf, whole plant tissue and grain collected from six field locations during the 1994 growing season using an Enzyme Linked Immuno-Sorbent Assay (ELISA) (Harlow and Lane, 1988). The six field sites established and conducted under GLP were as follows: Jerseyville, Illinois; Monmouth, Illinois; Johnston, Iowa; Sheldahl, Iowa; Windfall, Indiana; and York, Nebraska. The pedigree of the corn seed planted in the trial was BC1F2; resulting grain utilized in the analysis was BC1F3. The youngest whorl leaf was collected from 12 plants (V4 - V6 stage) and pooled. There was one pooled leaf sample of each line from each site. The leaf pool was processed to a fine powder and an aliquot removed for analysis. Two entire whole plants (root, stalk, leaves, ears, tassels) were harvested at one site. The entire plant was processed to a fine powder and an aliquot removed for analysis. There was one pooled grain sample of each line from each site, consisting of grain from up to approximately 100 - 170 ears. A sample of the pooled grain collected at each site was processed to a fine powder and an aliquot removed for analysis. The estimated expression levels are shown in Table IV.1.

Table IV.1 Summary of Specific Protein Levels Measured in Tissues of MON 802 Corn Plants

	CryIA(b)	CP4 EPSPS	GOX
	-µg/g fresh weight-		
MON 802			
Leaf[1]	9.55	26.99	10.18
Whole Plant[2]	1.35	1.85	1.68
Grain[1]	3.20	2.27	N.D.[3]

1: Values are means calculated across six sites.
2: Values are means calculated from the analysis of two replicate plant samples from one site.
3: Not detected.

As seen above, expression levels of CryIA(b), CP4 EPSPS and GOX proteins are low in corn leaf, whole plant and grain tissues from the insect-protected Roundup Ready line MON 802, but sufficient to produce the desired phenotype.

E. Effects of Insect-protected Corn on Non-target Organisms

1. Non-target insects

There is extensive information on the lack of non-target effects from microbial preparations of *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) containing the *B.t.k.* proteins, including the CryIA(b) protein. The literature has established that the *B.t.k.* proteins:

- are extremely selective for the lepidopteran insects (MacIntosh *et al.*, 1990; Klausner, 1984; Aronson *et al.*, 1986; Dulmage, 1981; Whitely and Schnepf, 1986);
- bind specifically to receptors on the mid-gut of lepidopteran insects (Wolfersberger *et al.*, 1986; Hofmann *et al.*, 1988a; Hofmann *et al.*, 1988b; Van Rie *et al.*, 1989; Van Rie *et al.*, 1990); and
- have no deleterious effect on beneficial/non-target insects, including predators and parasitoids of lepidopteran insect pests or honeybee (*Apis mellifera*) (Flexner *et al.*, 1986; Krieg and Langenbruch, 1981; Cantwell *et al.*, 1972; EPA, 1988; Vinson, 1989; Melin and Cozzi, 1989).

The chapters by Vinson (1989) and Melin and Cozzi (1989) provide comprehensive reviews of the extensive literature that has established the safety of the *B.t.k.* microbes and encoded proteins to an array of beneficial insects.

In addition, separate studies were undertaken to assess the potential toxicity of the CryIA(b) protein to other non-target insects.

a. Honey bee larvae and adults
These studies were undertaken to assess the potential toxicity of the CryIA(b) trypsin-resistant core protein to larvae and adult honey bee (*Apis mellifera* L.), a beneficial insect pollinator. The maximum nominal CryIA(b) protein concentration tested was greater than 10 times the estimated LC_{50} sensitivity of several target pest Lepidoptera to the CryIA(b) protein. The LC_{50} for the CryIA(b) protein in larval and adult honey bee is greater than 20 ppm. The no observed effect level was 20 ppm (Maggi and Sims, 1994a, 1994b).

b. Green lacewing
This study was undertaken to assess the potential toxicity of the CryIA(b) trypsin-resistant core protein to green lacewing larvae (*Chrysopa carnea*), a beneficial predaceous insect commonly found in corn and other cultivated plants. There was no evidence that green lacewing larvae were adversely effected when fed moth eggs coated with a nominal concentration of 16.7 ppm CryIA(b) protein for seven days. Under the conditions of the test, the LC50 was greater than 16.7 ppm CryIA(b) protein (Hoxter and Lynn, 1992a).

c. Parasitic hymenoptera
This study was undertaken to assess the potential toxicity of the CryIA(b) trypsin-resistant core protein to parasitic Hymenoptera (*Brachymeria intermedia*), a beneficial parasite of the housefly (*Musca domestica).* Parasitic Hymenoptera exposed to activated CryIA(b) protein at a concentration of 20 ppm in honey/water solution for thirty days did not exhibit treatment related mortality or signs of toxicity. The LC_{50} for CryIA(b) protein in parasitic Hymenoptera is greater than 20 ppm. The no-observed effect level was 20 ppm (Hoxter and Lynn, 1992c).

d. Ladybird beetle
This study was undertaken to assess the potential toxicity of CryIA(b) trypsin-resistant core protein to ladybird beetles (*Hippodamia convergens*), a beneficial predaceous insect which feeds on aphids and other plant insects commonly found on stems and foliage of weeds and cultivated plants. Ladybird beetles exposed to activated CryIA(b) protein at a test concentration of 20 ppm in a honey/water solution for nine days did not exhibit treatment related mortality or signs of toxicity. The LC_{50} for CryIA(b) protein in ladybird beetles is greater than 20 ppm. The no-observed effect level was 20 ppm (Hoxter and Lynn, 1992b).

2. Non-Target wildlife and fish
It is unlikely that fish in their natural environment would be exposed to corn seed. Based on the historical data demonstrating the safety of *B.t.* proteins to fish and the unlikely event of exposure, no adverse effects are expected to fish from the use of the corn containing the CryIA(b) protein.

3. Impact on endangered species
No endangered or threatened lepidopteran insects, as listed in 50CFR 17.11 and 17.12, feed on corn plants.

F. Environmental Fate of the CryIA(b) Protein

Previous work has demonstrated the rapid loss of insecticidal activity of *Bacillus thuringiensis* protein crystals (active ingredient) derived from microbial preparations of lepidopteran-active species when incubated in the soil (Palm *et al.*, 1994; Pruett *et al.*, 1980; West, 1984). The CryIA(b) protein in insect-protected Roundup Ready corn line MON 802 is present at low levels in the plant tissue remaining in the field after the harvest of the seed or silage. This corn plant material may be tilled into the soil or remain on the soil surface as is typically observed in zero tillage systems. The environmental fate of the CryIA(b) protein was determined by measuring the rate at which the bioactivity of the CryIA(b) protein dissipated when added to soil as the purified protein and as a component of insect-protected corn tissue.

Studies examined the dissipation rate of the CryIA(b) protein from three systems: 1) insect-protected corn tissue without contact with soil, 2) insect-protected corn tissue mixed into soil, and 3) purified CryIA(b) protein mixed into soil. The levels incorporated into the soil were greater than three fold higher than the maximum concentration expected under field conditions. CryIA(b) protein, added to soil as a component of tissue from insect-protected corn had an estimated DT_{50} of 1.6 days. Bioactivity of insect-protected corn tissue, incubated without soil contact, had an estimated DT_{50} of 25.6 days. Purified CryIA(b) protein, mixed into the soil, had an estimated DT_{50} of 8.3 days. This rate of dissipation of insecticidal activity is comparable to that observed with microbial *B.t.* products (Sims and Sanders, 1995).

Therefore, results of this study suggest that the CryIA(b) protein, as a component of post-harvest insect-protected corn plants, will dissipate readily on the surface of (e.g. no-till), or when cultivated into the soil. The measured half-life of the purified *B.t.k.* protein in soil is comparable to that measured for the microbial *B.t.k.* preparations (Palm *et al.*, 1994; Pruett *et al.*, 1980; West, 1984).

G. Weediness Potential of Insect-protected Corn

The potential for pollen transfer from corn to other species and for the insect-protected corn to become a weed or pest is addressed in Part II of this document in the paper "Potential for Outcrossing and Weediness of Genetically Modified Insect Resistant Corn", by Arnel R. Hallauer, Ph.D., Department of Agronomy, Iowa State University.

Based upon the report cited above, an extensive review of literature, and a history of nonregulated status for genetically modified corn phenotypes (96-

017-01p, 95-195-01p, 95-145-01p, 95-093-01p, 94-357-01p and 93-319-01p) outcrossing from corn to other species and for the insect-protected corn to become a weed or pest is not considered possible in the United States.

1. Outcrossing to wild *Zea* species

For gene flow to occur via normal sexual transmission, certain conditions must exist: (1) the two parents must be sexually compatible, (2) their fecundity must coincide, (3) a suitable pollen vector must be present and capable of transferring pollen between the two parents, and (4) resulting progeny must be fertile and ecologically fit for the environment in which they are situated.

Corn and annual teosinte (*Zea mays* ssp. *mexicana* Schrad.) are genetically compatible, wind pollinated, and in areas of Mexico and Guatemala they freely hybridize when in proximity to each other. Corn easily crosses with teosinte, but teosinte is not present in the U.S. other than occasional botanical garden example specimens of teosinte. These specimens would flower at the same time as corn (due to photoperiod reaction) only if they were subject to artificial daylength-shortening for several weeks at a time (Wilkes, 1967). Differences in factors such as flowering time, geographical separation and developmental factors make nature crosses in the United States speculative.

The habitat preferences of *Tripsacum*, another closely related genus, are similar to those of teosinte with twelve of the sixteen species native to Mexico and Guatemala. *Tripsacum floridanum* (Florida Gamagrass) is native to the southern tip of Florida. Outcrossing with *Tripsacum* species is not known to occur in the wild and only with extreme difficulty can corn be crossed with *Tripsacum*. Further, the offspring of this cross show varying levels of sterility (Galinat, 1988; Manglesdorf, 1974; Russell and Hallauer, 1980). No cases of gene flow between corn and its wild relatives are known in the United States.

2. Outcrossing to cultivated *Zea* varieties

Gene exchange between cultivated corn and genetically modified corn would be similar to what naturally occurs at the present time. Wind-blown pollen would move about among plants within the same field and among plants in nearby fields. Free flow of genes would occur similar to what occurs in cultivated corn. The production of the CryIA(b) protein in resulting seed would not be an issue due to the safety demonstrated for the insect-protected corn.

3. Weediness or pest potential of corn

Modern corn cannot survive as a weed because of past selection in the evolution of corn. In contrast with weedy plants, the corn ear is enclosed with husks. Consequently, seed dispersal of individual kernels naturally does not occur because of the structure of ears of corn. However, even as individual kernels of corn are distributed in the fields and main avenues of travel from the field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities, volunteer corn is not found growing in fence rows, ditches, and road sides as a weed (Hallauer, Part II of this

document. The Corn Family). Further, although corn seed can overwinter into a crop rotation with soybeans, mechanical and chemical measures are utilized for control. In neither instance (natural or mechanical harvesting) does corn become a troublesome weed. Corn cannot survive without human assistance and is not capable of surviving as a weed (Galinat, 1988; Rissler and Mellon, 1993).

4. Transfer of genetic information to species to which it cannot interbreed

As stated in the USDA's Interpretative Ruling on Calgene, Inc., Petition for Determination of Regulatory Status (FR 57, No.202, pp 47608-47616, October 19, 1992) "There is no published evidence for the existence of any mechanism, other than sexual crossing" by which genes can be transferred from a plant to other organisms. Evidence presented in the Calgene petition and supplementary information and summarized in the FR Notice suggests that, based on limited DNA homologies, transfer from plants to microorganisms may have occurred in evolutionary time over many millennia. Even if such transfer were to take place, transfer of the *cryIA(b)*, CP4 EPSPS or *gox* genes to a microbe would not pose any plant pest risk. As described earlier in this document, the *cryIA(b)* gene which was transferred to corn was isolated from *Bacillus thuringiensis* subsp. *kurstaki*, a commonly occuring soil microbe. The CP4 EPSPS gene, isolated from an *Agrobacterium* sp., and the *gox* gene, isolated from *Achromobacter*, are also representative of naturally occurring soil microbes. Based on these considerations, transfer to microbes or other living species in nature is quite unlikely and of no significant consequence from a plant pest point of view.

H. Field Germination Results

Field germination data was collected in 1994 to compare the survival characteristics of the insect-protected Roundup Ready corn line MON 802 to its parental control. Seed germination of MON 802 and its parental control was examined at five field locations (USDA Notification 94-060-03N).

Table IV.2 Field Germination Results

	MON 802			Control		
Location	Kernels planted	Seedlings emerged	% germ.	Kernels planted	Seedlings emerged	% germ
Jerseyville, IL	360	283	79	360	284	79
Monmouth, IL	360	338	94	360	354	98
Johnston,IA	264	259	98	264	257	97
Windfall, IN	272	252	93	272	254	93
York, NE	264	239	91	264	225	85
Ave.			91%			91%

Results showed that all seed samples demonstrated high rates of germination with no differences observed between corn line MON 802 and its respective control under a variety of environmental conditions.

I. Disease and Pest Susceptibilities

Insect-protected Roundup Ready corn line MON 802 has been tested in the United States since 1993. Monitoring for the disease and insect susceptibility of this line when compared to non-transgenic plants was performed each year at the sites listed in Table IV.4. No differences in agronomic quality, disease, or insect susceptibility other than the desired activity on certain lepidopteran insects including European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*) and corn earworm (*Heliothis zea*) were detected between this line and non-genetically modified plants.

Diseases observed included common maize rust (*Puccinia sorghi*), southern corn rust (*Puccinia polysora*), common corn smut (*Ustilago maydis*), Fusarium stalk rot (*Fusarium moniliforme*), Gibberella stalk rot (*Gibberella zea*e), northern leaf blight (*Exserohilum turcicum),* southern leaf blight (*Bipolaris maydis*), gray leaf spot (*Cercospora zeae-maydis*), maize dwarf mosaic virus (MDMV) and maize streak virus (MSV).

Insects observed in the plot areas included European corn borer, southwestern corn borer, corn earworm, southern cornstalk borer (*Diatraea crambidoides*), western corn rootworm (*Diabrotica virgifera*), northern corn rootworm (*Diabrotica barberi*), corn flea beetles (*Chaetocnema* sp.), lacewing (*Chrysopa* sp.), black cutworm (*Agrotis ipsilon*), billbugs (*Sphenophorus* sp.), *Orius insidiosus*, ladybird beetle (*Hippodamia convergens*), grasshoppers (*Melanoplus* sp.), leaf hoppers (*Trigonotylus* and other species), thrips (*Anaphothrips* and *Frankliniella* sp.), and mites (*Tetranychus* and *Oligonychus* sp.).

J. Compositional Analysis of Insect-Protected Roundup Ready Corn Line MON 802

Insect-protected Roundup Ready corn line MON 802, and the control line, MON 818 were grown in 1994 at six field locations and the harvested grain analyzed to establish that the composition of these corn lines is substantially equivalent to that of corn varieties grown commercially. Corn grain was collected from the following sites: Jerseyville, IL; Monmouth, IL; Johnston, IA; Sheldahl, IA; York, NE; and Windfall, IA. Each field site is located in the major corn growing region (e.g. Corn Belt) of the United States and is representative of various local growing practices where insect-protected Roundup Ready corn varieties are suitable as commercial products. The pedigree of the corn seed planted in the trial was BC1F2; resulting grain utilized in the analysis was BC1F3.

The compositional parameters measured included protein, fat, ash, crude fiber, moisture, calcium and phosphorus (AOAC methods, 1990). The values reported for the compositional analyses performed at Corning Hazleton, Inc. (Madison, WI), were expressed as percent dry weight of the sample correcting for the measured moisture content. The mean values for each component for each test sample across all sites was calculated. These values were calculated from the values measured for each sample, one from each of six sites. The range represents the minimum and maximum values from the analyses of samples across all sites (Sanders *et al.,* 1996). The experimental values obtained for corn line MON 802 were comparable to the control line, MON 818, as well as to published literature ranges (Watson, 1982, 1987; Jugenheimer, 1976).

The levels of the major components of corn grain (protein, fat, ash, crude fiber, carbohydrate, calories and moisture) were determined for grain and are summarized in Table IV.3. The inorganic components, calcium and phosphorus, were also measured and reported. The levels of each of these components were similar for the corn line MON 802 and the parental control line MON 818 and comparable to the published literature (Watson, 1987; Jugenheimer, 1976).

Table IV.3 Summary of Proximate, Calcium and Phosphorus Analysis of Corn Grain from Line MON 802[a]

Characteristic	MON 818 Control Mean[b] (Range)[c]	MON 802 Test Mean[b] (Range)[c]	Literature (Range)
Protein	12.8 (11.7-13.6)	12.9 (11.8-13.7)	(6.0-12.0)[d] (9.7-16.1)[e]
Fat	2.9 (2.6-3.2)	3.1 (2.8-3.2)	(3.1-5.7)[d] (2.9-6.1)[e]
Ash	1.5 (1.5-1.6)	1.6 (1.5-1.8)	(1.1-3.9)[d]
Crude Fiber	2.4 (2.3-2.5)	2.4 (2.1-2.6)	(2.0-5.5)[d]
Carbohydrate	82.7 (81.7-83.8)	82.4 (81.5-83.2)	Not reported
Calories/100g	409 (406-410)	409 (409-410)	Not reported
Calcium %	0.003 (0.003-0.004)	0.003 (0.003-0.003)	(0.01-0.1)[d]
Phosphorus %	0.348 (0.327-0.363)	0.336 (0.321-0.356)	(0.26-0.75)[d]
Moisture %	12.0 (10.6-14.2)	12.6 (11.2-14.8)	(7-23)[d]

a: Percent dry weight of sample, except for moisture.
b: Value reported is mean of six samples, one from each field site.
c: Range denotes the lowest and highest individual values across sites for each line.
d: Watson, 1987.
e: Jugenheimer, 1976.

The FDA Food Policy recommends that key compositional components of genetically modified plant varieties be assessed prior to commercial introduction. Monsanto has performed extensive analytical studies to compare the composition of insect-protected Roundup Ready corn line MON 802 to the control line. The compositional data demonstrates that the grain from this line is substantially equivalent to the parental variety and other corn varieties grown commercially. The absence of unexpected or unintended effects due to the expression of the CryIA(b), CP4 EPSPS and GOX proteins in the corn line is demonstrated by the establishment that the host organism, corn, has a safe history of use and extensive compositional analysis of this corn line with comparison to the control line and published ranges for other corn varieties.

Monsanto has completed its consultation on the food and feed safety of insect-protected Roundup Ready corn line MON 802 as initiated with the FDA under their May 29, 1992 policy statement concerning foods derived from new plant varieties (Croon and Sanders, 1996).

Table IV.4 Disease and Insect Susceptibility of Insect-protected Roundup Ready Corn Line MON 802.

Line/Year/site/ USDA permit/notification no.	Difference in susceptibility vs. non-modified corn plants* Disease	Insect
Insect-Protected Roundup Ready Corn Line MON 802		
1993		
Kekaha, HI (4/93) (92-209-02)	no	no
Kekaha, HI (8/93) (92-209-02)	no	no
Monmouth, IL (93-012-04)	no	no
Jerseyville, IL (93-012-04)	no	no
Salinas, PR (93-144-02N)	no	no
Kekaha, HI (11/93) (93-245-02N)	no	no
Loxley, AL (93-250-04N)	no	no
Kaunakakai, HI (11/93) (93-258-04N)	no	no
Kaunakakai, HI (12/93) (93-279-04N)	no	no
Kaunakakai, HI (12/93) (93-308-02N)	no	no
1994		
Kekaha, HI (4/94) (93-245-02N)	no	no
Kaunakakai, HI (5/94) (93-279-04N)	no	no
Kaunakakai, HI (8/94) (93-279-04N)	no	no
Isabela, PR (2/94) (93-306-04N)	no	no
Isabela, PR (6/94) (93-306-04N)	no	no
Kaunakakai, HI (1/94) (93-316-04N)	no	no
Kaunakakai, HI (5/94) (93-316-04N)	no	no
Kaunakakai, HI (9/94) (93-316-04N)	no	no
Kunia, HI (1/94) (93-354-06N)	no	no
Kaunakakai, HI (3/94) (94-026-04N)	no	no
Santa Isabel, PR (4/94) (94-026-04N)	no	no
Platteville, WI (94-033-04N)	no	no
Jerseyville, IL (94-060-03N)	no	no
Monmouth, IL (94-060-03N)	no	no
Farmer City, IL (94-074-12N)	no	no

Table IV.4 Disease and Insect Susceptibility of Insect-protected Roundup Ready Corn Line MON 802. (continued)

Line/Year/site/ USDA permit/notification no.	Difference in susceptibility vs. non-modified corn plants* Disease	Insect
1994 (contin.)		
Shirley, IL (94-074-12N)	no	no
Clinton, IL (94-074-14N)	no	no
Henrietta, MO (94-074-14N)	no	no
Waterloo, NE (94-074-14N)	no	no
Jerseyville, IL (94-082-03N)	no	no
Monmouth, IL (94-082-03N)	no	no
Phillips, NE (94-082-09N)	no	no
Washington, IA (94-082-09N)	no	no
St. Joseph, IL (94-082-09N)	no	no
Aurora, IL (94-082-10N)	no	no
Sugar Grove, IL (94-082-10N)	no	no
Monticello, IL (94-082-10N)	no	no
Grinnell, IA (94-082-10N)	no	no
Covington, OH (94-082-10N)	no	no
Carrollton, MO (94-082-10N)	no	no
Champaign, IL (94-082-05N)	no	no
Franklin, IN (94-082-04N)	no	no
Williamsburg, IA (94-082-04N)	no	no
Stonington, IL (94-083-02N)	no	no
Wood River, NE (94-083-03N)	no	no
Slater, IA (94-083-03N)	no	no
Stanton, MN (94-083-04N)	no	no
Kaunakakai, HI (7/94) (94-171-05N)	no	no
Santa Isabel, PR (8/94) (94-171-05N)	no	no
Kaunakakai, HI (11/94) (94-279-03N)	no	no
Santa Isabel, PR (11/94) (94-279-03N)	no	no
Center Point, IA (94-024-03N)	no	no
Vinton, IA (94-024-03N)	no	no
Algona, IA (94-024-03N)	no	no
Callendar, IA (94-024-03N)	no	no
Johnston, IA (94-024-03N)	no	no

Table IV.4 Disease and Insect Susceptibility of Insect-protected Roundup Ready Corn Lines MON 802. (continued)

Line/Year/site/ USDA permit/notification no.	Difference in susceptibility vs. non-modified corn plants* Disease	Insect
1994 (contin.)		
Sheldahl, IA (94-024-03N)	no	no
Melbourne, IA (94-024-03N)	no	no
Scranton, IA (94-024-03N)	no	no
Seymour, IL (94-024-04N)	no	no
Macomb, IL (94-024-04N)	no	no
Dover, IL (94-024-04N)	no	no
Shelbyville, IL (94-024-04N)	no	no
Long Point, IL (94-024-04N)	no	no
Wheatfield, IN (94-024-10N)	no	no
Tipton, IN (94-024-10N)	no	no
York, NE (94-024-11N)	no	no
Janesville, WI (94-024-06N)	no	no
Mankato, MN (94-024-08N)	no	no
Breckenridge, MI (94-024-07N)	no	no
Lancaster, PA (94-024-09N)	no	no
Huron, SD (94-024-05N)	no	no
Kekaha, HI (7/94) (94-159-01N)	no	no
Seven Springs, NC (94-024-02N)	no	no
Milton, WI (94-025-05N)	no	no
Lamar, CO (94-038-01N)	no	no
Parkersburg, IA (94-038-01N)	no	no
Danville, IA (94-038-01N)	no	no
Geneseo, IL (94-038-01N)	no	no
Carlyle, IL (94-038-01N)	no	no
Monmouth, IL (94-038-01N)	no	no
Noblesville, IL (94-038-01N)	no	no
Elk City, KS (94-038-01N)	no	no
La Center, KY (94-038-01N)	no	no
Conklin, MI (94-038-01N)	no	no
East Grand Forks, MN (94-038-01N)	no	no
Hills, MN (94-038-01N)	no	no

Table IV.4 Disease and Insect Susceptibility of Insect-protected Roundup Ready Corn Line MON 802. (continued)

Line/Year/site/ USDA permit/notification no.	Difference in susceptibility vs. non-modified corn plants* Disease	Insect
1994 (contin.)		
Macon, MO (94-038-01N)	no	no
York, NE (94-038-01N)	no	no
Osceola, NE (94-038-01N)	no	no
New Holland, OH (94-038-01N)	no	no
Germansville, PA (94-038-01N)	no	no
Renner, SD (94-038-01N)	no	no
Levelland, TX (94-038-01N)	no	no
Webster City, IA (94-038-01N)	no	no
Monmouth, IL (94-055-18N)	no	no
Ames, IA (94-055-18N)	no	no
Garden City, KS (94-055-18N)	no	no
Mead, NE (94-055-18N)	no	no
Hastings, NE (94-055-18N)	no	no
Concord, NE (94-055-18N)	no	no
St. John, KS (94-055-18N)	no	no
Rockport, MO (94-055-18N)	no	no
Steele, MO (94-055-18N)	no	no
Rockport, MO (94-055-18N)	no	no
McLean, VA (94-074-09N)	no	no
Mankato, MN (94-083-04N)	no	no
Kaunakakai, HI (11/94) (94-265-01N)	no	no
Kaunakakai, HI (11/94) (94-266-01N)	no	no
1995	no	no
Renner, SD (94-038-01N)	no	no
Levelland, TX (94-038-01N)	no	no
Webster City, IA (94-038-01N)	no	no
Monmouth, IL	no	no
Santa Isabel, PR (12/95) (94-279-03N)	no	no
Waterloo, NE (95-072-03N)	no	no

Table IV.4 Disease and Insect Susceptibility of Insect-protected Roundup Ready Corn Line MON 802. (continued)

Line/Year/site/ USDA permit/notification no.	Difference in susceptibility vs. non-modified corn plants* Disease	Insect
1995 (contin.)		
Henrietta, MO (95-072-03N)	no	no
Clinton, IL (95-072-03N)	no	no
Platteville, WI (95-072-03N)	no	no
Galena, MD (95-075-01N)	no	no
Ames, IA (95-075-01N)	no	no
Garden City, KS (95-075-01N)	no	no
Scottsburg, IN (95-075-01N)	no	no
Battle Ground, IN (95-075-01N)	no	no
St. John, KS (95-075-01N)	no	no
Ashland, KS (95-075-01N)	no	no
Champaign, IL (95-075-01N)	no	no
Alhambra, IL (95-075-01N)	no	no
Monmouth, IL (95-083-10N)	no	no
Ames, IA (95-083-10N)	no	no
Mead, NE (95-083-10N)	no	no
Jerseyville, IL (95-083-10N)	no	no
Benton, IA (95-083-10N)	no	no
Monmouth, IL (95-087-10N)	no	no
Kekaha, HI (6/95) (94-365-03N)	no	no
Carrollton, MO (95-039-09N)	no	no
Janesville, WI (95-039-12N)	no	no
Huron, SD (95-039-11N)	no	no
York, NE (95-039-16N)	no	no
Lexington, KY (95-037-07N)	no	no
Algona, IA (95-074-11N)	no	no
Callendar, IA (95-074-11N)	no	no
Woodward, IA (95-074-11N)	no	no
Martensdale, IA (95-074-11N)	no	no
Sheldahl, IA (95-074-11N)	no	no
Johnston, IA (95-074-11N)	no	no
Marion, IA (95-074-11N)	no	no
Vinton, IA (95-074-11N)	no	no
Mankato, MN (95-039-14N)	no	no
Windfall, IN (95-039-15N)	no	no
Breckenridge, MI (95-039-13N)	no	no
Winterville, NC (95-032-02N)	no	no

*insects as identified in Section IV.I

K. References

Anderson. K.S. and K.A. Johnson. 1990. Kinetic and structural analysis of enzyme intermediates: Lessons from EPSP synthase. Chem. Rev. 90:1131-1149.

AOAC Methods. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia.

Aronson, A.I., W. Backman, and P. Dunn. 1986. *Bacillus thuringiensis* and related insect pathogens. Microbiol. Rev. 50:1-24.

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

Barry, G.F., M.L. Taylor, S.R. Padgette, K.H. Kolacz, L.E. Hallas, G. della-Cioppa, and G.M. Kishore. 1994. Cloning and Expression in *Escherichia coli* of the Glyphosate-to-Aminomethylphosphonic Acid Degrading Activity from *Achromobacter* sp. strain LBAA. Monsanto Technical Report MSL-13245, St. Louis.

Bielot, H., Carey, P. R., Kaplan, H., Lessard, T., and Pozsgay, Z. 1989. "Facile preparation and characterization of the toxin from *Bacillus thuringiensis* var. *kurstaki*," Biochem. J. 260: 87-91.

Bulla, L.A., Kramer, K.J., and Davidson, L.I. 1977. Characterization of the entomocidal parasporal crystal of *Bacillus thuringiensis*. J. Bacteriol. 130:375-383.

Cantwell, G.E., T. Lehnert, and J. Fowler. 1972. Are biological insecticides harmful to the honey bees. Am. Bee J. 112: 294-296.

Cole, D.J. 1985. Mode of action of glyphosate - a literature analysis. pp. 48 - 74. *In* The Herbicide Glyphosate. Grossbard, E., and D. Atkinson (eds.), Buttersworths, Boston.

Croon, K.A., P.R. Sanders, P.J. Keck, T.C. Lee, and G.B. Parker. 1995. Petition for Determination of Nonregulated Status: Insect-protected Corn (*Zea mays* L.) with the cryIA(b) Gene from *Bacillus thuringiensis* subsp. *kurstaki*. USDA Petition no. 95-093-01P.

Croon, K.A. and P.R. Sanders. 1996. Safety, Compositional and Nutritional Aspects of Insect-Protected Roundup Ready Corn Lines MON 802 and MON 805 and Roundup Ready Corn Lines MON 830, MON 831 and MON 832. FDA Summary.

della-Cioppa, G., Bauer, C.S., Klein, B.K., Shah, D.M., Fraley, R.T., and Kishore, G.M. 1986. Translocation of the Precursor of 5-Enolpyruvylshikimate-3-phosphate Synthase into Chloroplasts of Higher Plants *in vitro*. PROC. Natl., Acad. Sci. USA 83:6873-6877.

Dulmage, H.T. 1981. Microbial Control of Pests and Plant Diseases 1970 - 1980. (ed. Burges, H.D.) pp. 193-222, Academic Press, London.

EPA. 1988. Guidance for the reregistration of pesticide products containing *Bacillus thuringiensis* as the active ingredient. NTIS PB 89-164198.

Flexner, J.L., B. Lighthart, and B.A. Croft. 1986. The effects of microbial pesticides on non-target beneficial arthropods", Agric. Ecosys. Environ. 16:203-254.

Galinat, W.C. 1988. The Origin of Corn. pp. 1 - 31. *In* Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Gasser, C.S., J.A. Winter, C.M. Hironaka, and D.M. Shah. 1988. Structure, expression, and evolution of the 5-enolpyruvyl-shikimate-3-phosphate synthase genes of petunia and tomato. J. Biol. Chem. 263: 4280-4289.

Hallas, L.E., E.M. Hahn, and C. Korndorfer. 1988. Characterization of microbial traits associated with glyphosate biodegradation in industrial activated sludge. J. Industrial Microbiol. 3:377-385.

Harlow, E., and D. Lane. 1988. Immunoassay. Antibodies: A Laboratory Manual. Chapter 14:553-612.

Hofmann, C., Vanderbruggen, H. V., Hofte, H., Van Rie, J., Jansens, S., and Van Mellaert, H. 1988a. Specificity of *B. thuringiensis* delta-endotoxins is correlated with the presence of high affinity binding sites in the brush border membrane of target insect midguts. *Proc. Natl. Acad. Sci. USA* 85: 7844-7848.

Hofmann, C., Lüthy, P., Hutter, R. and Pliska, V. 1988b. Binding of the delta endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). *Eur. J. Biochem.* 173:85-91.

Hoxter, K.A. and S.P. Lynn. 1992a. Activated *Btk* HD-1 Protein: A Dietary Toxicity Study with Green Lacewing Larvae. An unpublished study conducted by Monsanto Company. EPA MRID 43468003.

Hoxter, K.A. and S.P. Lynn. 1992b. Activated *Btk* HD-1 Protein: A Dietary Toxicity Study with Ladybird Beetles. An unpublished study conducted by Monsanto Company. EPA MRID 43468005.

Hoxter, K.A. and S.P. Lynn. 1992c. Activated *Btk* HD-1 Protein: A Dietary Toxicity Study with Parasitic Hymenoptera (*Brachymeria intermedia*). An unpublished study conducted by Monsanto Company. EPA MRID 43468004.

Huber, H.E. and Lüthy, P. 1981. *Bacillus thuringiensis* delta-endotoxin: composition and activation. *In* Pathogenesis of Invertebrate Microbial Diseases. E.W. Davidson, ed. pp. 209-234, Allanheld, Osmun Publishers, New Jersey, U.S.A.

Ignoffo, C.M. 1973. Effects of entomopathogens on vertebrates. *Ann. N.Y. Acad. Sci.* 217:144-172.

Joos, S., B. Lambert, F. Leyns, A. DeRoeck, and J. Swing. 1988. Inventory of natural rhizobacterial populations from different crop plants. pp. 139-142. *In* The Release of Genetically-engineered Micro-organisms. M. Sussman, C.H. Collins, F.A. Skinner, and D.E. Stewart-Tull, eds. Academic Press, London.

Jugenheimer, R.W. 1976. Corn Improvement, Seed Production, and Uses. John Wiley & Sons, Inc. New York, New York, USA.

Kersters, K. and J. De Ley. 1984. pp. 244-248. *In* Bergey's Journal of Systematic Bacteriology, Vol. 1. Kreig, N.R. and J.G. Holt, eds. Williams and Wilkins, Baltimore.

Krieg, A. and G.A. Langenbruch. 1981. Susceptibility of arthropod species to *Bacillus thuringiensis*. *In* Microbial Control of Pests and Plant Diseases, (ed. H. D. Burges) pp. 837-896. Academic Press, London.

Klausner, A. 1984. Microbial insect control. Bio/Technology 2:408-419.

Levin, J.G. and D.B. Sprinson. 1964. The enzymatic formation and isolation of 3-enolpyruvylshikimate-5-phosphate. J. Biol. Chem. 239: 1142-1150.

MacIntosh, S.C., T.B. Stone, S.R. Sims, P. Hunst, J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff, and R.L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Insect Path. 56:258-266.

Maggi, V.L. and S.R. Sims. 1994a. Evaluation of the Dietary Effects of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Adults. An unpublished study conducted by Monsanto Company. EPA MRID 43439203.

Maggi, V.L. and S.R. Sims. 1994b. Evaluation of the Dietary Effects of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Larvae. An unpublished study conducted by Monsanto Company. EPA MRID 43439202.

Manglesdorf, Paul C. 1974. Corn - Its Origin, Evolution, and Improvement. Harvard Univ. Press. Cambridge, MA.

Melin, B.E. and E.M. Cozzi. 1989. *In* "Safety of Microbial Insecticides", (eds. Laird, M., Lacey, L.A. and Davidson, E.W.) pp. 150-167. CRC Press, Boca Raton, FL.

Naylor, M. 1992. Acute Oral Toxicity Study of *Btk* HD-1 Tryptic Core Protein in Albino Mice. An unpublished Monsanto study. EPA MRID 43468001.

Noteborn, H. 1994. Safety assessment of genetically modified plant products. Case study: *Bacillus thuringiensis*-toxin tomato. *In* Biosafety of Foods Derived by Modern Biotechnology. Nika Rack, ed. BATS, Agency for Biosafety Research and Assessment of Technology Impacts, Basel, Switzerland.

Padgette, S.R., M.L. Taylor, G.F. Barry, L.A. Harrison, T. Huber, and G.M. Kishore. 1994. Characterization of Glyphosate Oxidoreductase. Monsanto Technical Report MSL-13234, an unpublished study conducted by Monsanto Company. EPA MRID no. 43903701.

Padgette, S.R., G. della-Cioppa, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1989. Selective herbicide tolerance through protein engineering. Cell Culture and Somatic Cell Genetics of Plants 6: 441-476.

Padgette, S.R., D.B. Re, C.S. Gasser, D.A. Eichholtz, R.B. Frazier, C.M. Hironaka, E.B. Levine, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1991. Site-directed mutagenesis of a conserved region of the 5-enolpyruvylshikimate-3-phosphate synthase active site. Journal of Biological Chemistry 266, No. 33, 22364-22369.

Padgette, S.R., C.E Smith, Q.K. Huynh, and G.M. Kishore. 1988. Arginine chemical modification of Petunia *hybrida* 5-enolpyruvylshikimate-3-phosphate synthase. Arch. Biochem. and Biophysics. 266:254-262.

Palm, C.J., K. Donegan, D. Harris, and R.J. Seidler. 1994. Quantification in soil of *Bacillus thuringiensis* var. *kurstaki* delta-endotoxin from transgenic plants. Molecular Ecology 3, 145-151.

Pruett, C., H.D. Burges, and C.H. Wyborn. 1980. Effect of exposure to soil on potency and spore viability of *Bacillus thuringiensis*. J. Invert. Pathol. 35: 168-174.

Ream, J.E. 1994. Assessment of the *In Vitro* Digestive Fate of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein. Study Number 93-01-39-04, MSL-13425, an unpublished study conducted by Monsanto Company. EPA MRID no. 43439201.

Rissler, J. and M. Mellon. 1993. Perils Amidst the Promise: Ecological Risks of Transgenic Crops in a Global Market. Union of Concerned Scientists, Cambridge, MA. pp. 22.

Russell, W.A. and A.R. Hallauer. 1980. Corn. pp. 299 - 312. *In* Fehr, W.R. and Hadley, H.H., Eds. Hybridization of Crop Plants. American Society of Agronomy and Crop Science Society of America, Publishers, Madison, Wisconsin, USA.

Sacchi, V. F., Parenti, P., Hanozet, G. M., Giordana, B., Luthy, P. and Wolfersberger, M. G. 1986. "*Bacillus thuringiensis* toxin inhibits K+-gradient-dependent amino acid transport across the brush border membrane of *Pieris brassicae* midgut cells," *FEBS Lett.* 204: 213-218.

Sanders, P.R., Henning, D.M., and Groth, M.E. 1996. Compositional Analysis of Insect-Protected and Insect-Protected Roundup Ready™ Corn Lines from the 1994 U.S. Field Trials. Study Number 94-01-50-18, MSL-14326, an unpublished study conducted by Monsanto Company. MSL-14326.

Schulz, A. , A. Krüper, and N. Amrhein. 1985. Differential sensitivity of bacterial 5-enolpyruvylshikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiology Letters 28:297-301.

Shadduck, J.A. 1983. Some observations on the safety evaluation of nonviral microbial pesticides. *Bull. W.H.O.* 61:117-128.

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

Siegel, J.P. and Shadduck, J.A. 1989. Safety of microbial insecticides to vertebrates --humans. *In* Safety of Microbial Insecticides, pp.101-114. CRC Press, Inc., Florida, U.S.A.

Sims, S.R., and P.R. Sanders. 1995. Aerobic Soil Degradation of *Bacillus thuringiensis* var. *kurstaki* HD-1 Protein. An unpublished study conducted by Monsanto Company. EPA MRID 43533206.

Stallings, W.C., S.S. Abdel-Meguid, L.W. Lim, H.S. Shieh, H.E. Dayringer, N.K. Leimgruber, R.A. Stegeman, K.S. Anderson, J.A. Sikorski, S.R. Padgette, and G.M. Kishore. 1991. Structure and topological symmetry of the glyphosate target 5-enolpyruvylshikimate-3-phosphate synthase: A distinctive protein fold. Proc. Natl. Acad Sci. USA 88:5046-5050.

Steinrüken, H.C. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimate-3-phosphate synthase. Biochem. Biophys. Res. Comm. 94: 1207-1212.

Timko, M.P., Herdies, L., de Almeida, E., Cashmore, A.R., Leemans, J., and Krebbers, E. 1988. Genetic engineering of nuclear-encoded components of the photosynthetic apparatus in *Arabidopsis*. pp. 279-295. *In* "The Impact of Chemistry on Biotechnology," ACS Books.

Van Rie, J., S. Jansens, H. Höfte, D. Deghelle, and H. Van Mellaert. 1990. Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Appl. Environ. Microbiol. 56:1378-1385.

Van Rie, J., S. Jansens, H. Höfte, D. Degheele, and H. Van Mellaert. 1989. Specificity of *Bacillus thuringiensis* ∂-endotoxins, importance of specific receptors on the brush border membrane of the mid-gut of target insects. Eur. J. Biochem. 186:239-247.

Vinson, S.B. 1989. Potential impact of microbial insecticides on beneficial arthropods in the terrestrial environment. *In* Safety of Microbial Insecticides. (eds. Laird, M., L.A. Lacey, and E.W. Davidson.) pp. 43-64. CRC Press, Inc. Boca Raton, FL.

Watson, S.A. 1982. Corn: amazing maize. General properties. *In* CRC Handbook of Processing and Utilization in Agriculture. Volume II: Part 1 Plant Products. Wolff, I.A. ed. pp. 3-29. CRC Press, Inc. Boca Raton, FL.

Watson, Stanley A. 1987. Structure and Composition. Pg. 53-82. *In* Corn: Chemistry and Technology, S.A. Watson and P.E. Ranstead (eds). American Association of Cereal Chemists, Inc., Minnesota.

West, A.W. 1984. Fate of the insecticidal, proteinaceous parasporal crystal of *Bacillus thuringiensis* in soil. Soil Biol. Biochem. 16: 357-360.

Whitely, H.R. and H.E. Schnepf. 1986. The molecular biology of parasporal crystal body formation in *Bacillus thuringiensis*". Ann. Rev. Microbiol. 40:549-576.

Wilkes, H.G. 1967. Teosinte: The Closest Relative of Maize. Bussey Institution, Harvard Univ., Cambridge, MA.

Wolfersberger, M.G., Hofmann, C. and P. Lüthy. 1986. Interaction of *Bacillus thuringiensis* delta-endotoxin with membrane vesicles isolated from lepidopteran larval midgut, *In* bacterial protein toxins. Falmagne, P., Fehrenbech, F.J., Jeljaszewics, J., and M,. Thelestam, eds. Gustav Fischer, pp. 237-238, New York, New York, U.S.A.

Part V. Statement of Grounds Unfavorable

Monsanto Company knows of no unfavorable grounds associated with insect-protected Roundup Ready corn line MON 802. Therefore, on the basis of the substantial potential benefits to the grower, the environment, and the consumer, Monsanto requests that these corn lines no longer be regulated under 7 CFR part 340.6.

Appendix 1

USDA Field Trial Termination Reports

APHIS# 92-209-02
MON# 92-096

[Pioneer Breeding Nursery]
[Kauai, Hawaii]
4/93 Planting

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

Introgress transgenes into elite inbred lines through traditional backcross breeding techniques.

EXPERIMENT DESCRIPTION

The following lines were planted on 4/06/93 over an area of 0.2 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 540-02-2, 523-06-1, 546-09-1, *576-01-1*, *557-04-4*, 559-44-1, 576-01-10, 540-08-1, 550-02-1, 583-11-2, 475-05-1, 557-04-1, 570-22-1, *554-03-2*, 540-07-1, *654-04-1*, 540-02-1, 544-04-2, 560-01-1, 576-12-1, 575-31-2, 554-03-1, 503-03-1, 588-13-1, 559-16-1, 572-24-6, 555-06-1, *572-24-1*, 583-11-1, 584-02-2, 569-02-1, 578-05-2, 549-07-1, 559-66-1, 487-32-1, 572-17-1, 575-07-1, 548-16-3
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	618-46-1, 662-08-1, 600-13-1, *604-09-1*, *600-14-2*, 635-11-2, *599-04-2*, 600-15-1, 618-40-1, 600-01-2, 600-17-1, 680-04-1

ELISA, PCR, and/or glyphosate treatments were used to identify positive plants for backcrossing. Successful pollinations were made from selected lines.

A total of approximately 1475 ears were harvested 7/27/93 derived from the following lines (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 540-02-2, 523-06-1, 546-09-1, *576-01-1*, *557-04-4*, *554-03-2*, *654-04-1*, 544-04-2, 575-31-2, 554-03-1, 503-03-1, 588-13-1, *572-24-1*, 569-02-1, 559-66-1, 548-16-3
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	600-13-1, *604-09-1*, *600-14-2*, 635-11-2, *599-04-2*, 618-40-1, 600-17-1, 680-04-1

DISPOSAL

Plots were destroyed on 8/12/93 by burning crop residues, followed by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and B73, or a BC1F1 to elite lines. Variability in plant phenotype was consistent with these genetic backgrounds.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to recurrent parent plants. Fungicides were applied to all plots prophylactically.

Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to recurrent parent plants. Insecticides were applied to all plots prophylactically.

Weediness

All transgenic plants exhibited normal corn phenotypes that preclude shattering or other forms of seed dispersal prior to manual harvest. Husks remained over the ears, and seed remained attached to the cob. No evidence of seed dormancy or delayed germination was observed. Corn is unable to propagate vegetatively following maturity, and this characteristic appeared unchanged in these lines.

ISOLATION

All open-pollinated corn within 200 meters of the transgenic plots was destroyed.

VOLUNTEERS

Fields were alternately irrigated and disked to remove volunteer plants. Only a small fraction of the field was planted to transgenic plants, so it was not possible to discern whether observed volunteers were transgenic or not. Following the initial irrigation on 9/18/93, over 100 volunteer plants were observed. These were disked into the soil. Following the second irrigation in October, 1993, approximately 20 volunteer plants were observed. These were disked into the soil. Following the last irrigation in November, 1993, no volunteer plants were observed.

APHIS# 92-209-02
MON# 92-096

[Pioneer Breeding Nursery]
[Kauai, Hawaii]
8/93 Planting

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

Introgress transgenes into elite inbred lines through traditional backcross breeding techniques.

EXPERIMENT DESCRIPTION

The following lines were planted on 8/18/93 over an area of 0.4 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 540-02-2, 523-06-1, 546-09-1, *576-01-1*, *557-04-4*, *554-03-2*, *654-04-1*, 544-04-2, 575-31-2, 554-03-1, 503-03-1, 588-13-1, *572-24-1*, 569-02-1, 559-66-1, 548-16-3
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	600-13-1, *604-09-1*, *600-14-2*, 635-11-2, *599-04-2*, 618-40-1, 600-17-1, 680-04-1

ELISA, PCR, and/or glyphosate treatments were used to identify positive plants for backcrossing. Successful pollinations were made from selected lines.

A total of approximately 2350 ears were harvested 11/9/93 -11/11/93 derived from the following lines (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 540-02-2, 523-06-1, 546-09-1, *576-01-1*, *557-04-4*, *554-03-2*, *654-04-1*, 544-04-2, 575-31-2, 554-03-1, 588-13-1, *572-24-1*, 559-66-1, 548-16-3
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	600-13-1, *604-09-1*, *600-14-2*, 635-11-2, *599-04-2*, 618-40-1, 680-04-1

DISPOSAL

Plots were destroyed on 12/10/93 by burning crop residues, followed by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 or subsequent backcross derived from Hi-Type II and elite line recurrent parents. Variability in plant phenotype was consistent with these genetic backgrounds. Conversion to recurrent parent phenotype progressed similarly to programs involving non-transgenic source genes.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to recurrent parent plants. Fungicides were applied to all plots prophylactically.

Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to recurrent parent plants. Insecticides were applied to all plots prophylactically.

Weediness

All transgenic plants exhibited normal corn phenotypes that preclude shattering or other forms of seed dispersal prior to manual harvest. Husks remained over the ears, and seed remained attached to the cob. No evidence of seed dormancy or delayed germination was observed. Corn is unable to propagate vegetatively following maturity, and this characteristic appeared unchanged in these lines.

ISOLATION

All open-pollinated corn within 200 meters of the transgenic plots was destroyed.

VOLUNTEERS

Fields were alternately irrigated and disked to remove volunteer plants. Only a small fraction of the field was planted to transgenic plants, so it was not possible to discern whether observed volunteers were transgenic or not. Volunteer plants were observed after irrigations on 12/15/93, 1/10/94, 3/11/94, and 3/21/94. Volunteer plants were destroyed after the first 3 irrigations by disking, and after the last irrigation by Roundup applications. All plants died from the Roundup. Since most transgenic lines in this planting had at least some tolerance to Roundup, it is unlikely any of the final stand of volunteer plants was transgenic. This suggests that seed derived from transgenic plants did not exhibit increased survival relative to non-transgenic seed.

APHIS# 93-012-04
MON# 93-002R

Monsanto Trials
Monmouth, IL
Jerseyville, IL
Lockbourne, OH
93 Corn Belt Plantings

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVES

The trials conducted by Monsanto at the above locations had several objectives:

- Generate plant material and seed for regulatory submissions
  - ◊ Monmouth and Jerseyville
- Assess weed control efficacy using post-emergence applications of Roundup®
  - ◊ Lockbourne
- Assess efficacy of *in-planta Bt* in controlling artificial infestations of European Corn Borer (ECB), and determine genetic segregation
  - ◊ Monmouth and Jerseyville
- Assess efficacy to post-emergence applications of Roundup® of lines genetically modified with genes conferring tolerance to glyphosate, and determine genetic segregation
  - ◊ Monmouth and Jerseyville
- Produce seed for further testing and evaluation
  - ◊ Monmouth and Jerseyville

VECTORS AND LINES AT EACH LOCATION

The following three tables list by vector the lines evaluated at each location.

TABLE 1. Lines grown at Lockbourne, Ohio.

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	523-06-1, 572-05-1, 576-01-1, 576-01-2

TABLE 2. Lines grown at Monmouth, Illinois

Vector	Lines
PV-ZMBK03+PV-ZMGT03+PV-ZMGT05	387-04-1
PV-ZMBK10	631-03-1, 634-07-1, 634-11-1, 666-08-1, 714-05-1, 714-06-1, 714-55-1
PV-ZMBK13+PV-ZMGT08	455-09-3, 455-11-2, 462-05-4, 462-11-1
PV-ZMBK13+PV-ZMGT09	576-04-1, 576-04-5, 576-18-3, 576-22-2, 578-02-1, 579-11-1, 579-15-3, 579-15-4, 581-04-2
PV-ZMCT01(PV-ZMBK07+PV-ZMGT10)	475-05-1, 481-10-1, 487-46-1, 503-03-1, 512-01-1, 512-03-1, 512-04-1, 514-02-1, 523-01-1, 523-04-2, 523-06-1, 523-07-2, 523-09-1, 532-06-1, 538-02-1, 540-02-2, 540-05-1, 540-08-1, 544-03-2, 546-09-1, 548-08-1, 548-16-3, 549-10-1, 550-02-2, 554-03-1, 554-03-2, 555-06-1, 557-04-1, 557-04-4, 559-14-2, 559-16-1, 559-31-1, 559-39-1, 559-39-2, 559-41-1, 559-44-1, 559-52-1, 559-66-1, 559-80-1, 560-01-1, 569-02-1, 570-16-1, 570-22-1, 570-23-2, 570-23-3, 572-03-1, 572-04-1, 572-04-2, 572-16-1, 572-18-1, 572-24-1, 572-24-6, 574-02-1, 574-04-1, 574-04-2, 574-04-3, 575-03-1, 575-07-2, 575-07-3, 575-26-1, 575-30-1, 575-31-1, 575-31-2, 575-32-1, 576-01-1, 576-01-10, 576-01-4, 576-12-1, 578-05-2, 579-12-1, 583-11-1, 583-11-2, 584-01-1, 584-02-2, 591-01-1, 591-03-1, 591-03-2, 639-05-1, 639-11-1, 645-01-1, 647-03-2, 647-04-1, 647-07-4, 647-10-2, 647-10-3, 654-04-1, 657-22-1, 658-01-1, 658-06-1, 661-01-1, 703-02-1
PV-ZMCT02(PV-ZMBK15+PV-ZMGT03)	599-01-3, 599-04-2, 599-04-3, 600-01-2, 600-12-1, 600-12-4, 600-13-1, 600-14-1, 600-14-2, 600-15-1, 600-15-2, 600-16-1, 600-17-1, 604-05-1, 604-05-2, 604-06-2, 604-09-1, 604-09-3, 604-19-1, 604-24-1, 609-07-2, 610-02-1, 611-04-2, 615-07-1, 617-64-1, 618-40-1, 618-41-1, 618-46-1, 618-47-2, 618-51-1, 619-10-1, 627-08-1, 627-08-3, 634-19-1, 634-22-2, 635-01-1, 635-04-1, 635-11-1, 635-11-2, 662-06-1, 662-08-1, 667-05-1, 678-06-1, 680-04-1
PV-ZMCT04(PV-ZMGT13+PV-ZMGT05)	425-01-1, 425-01-2, 425-01-3, 425-02-1
PV-ZMCT05(PV-ZMBK13+PV-ZMGT05)	462-03-2, 462-03-3, 462-04-1
PV-ZMCT06(PV-ZMBK17+PV-ZMGT01)	756-04-1
PV-ZMCT07(PV-ZMGT03+PV-ZMGT05)	366-04-1, 370-09-1, 400-01-1, 400-04-2, 400-06-1, 402-08-1, 402-09-1, 423-06-1
PV-ZMCT08(PV-ZMBK07+PV-ZMGT01)	689-09-2, 692-07-1, 694-02-1, 694-06-1, 771-07-1
PV-ZMGT02+PV-ZMGT03	365-02-1
PV-ZMGT03+PV-ZMGT09	400-05-1
PV-ZMGT04+PV-ZMGT01	347-03-1

TABLE 3. Lines grown at Jerseyville, Illinois

Vector	Lines
PV-ZMBK03+PV-ZMGT01	347-01-1
PV-ZMBK10	631-03-1, 634-07-1, 634-11-1, 634-11-1, 666-08-1, 701-12-1, 714-05-1, 714-06-1, 714-55-1
PV-ZMBK10L	749-08-2, 749-09-1
PV-ZMBK12	727-04-1
PV-ZMBK12L	749-01-1
PV-ZMBK13+PV-ZMGT08	455-07-2, 455-09-3, 455-11-2, 462-05-1, 462-05-4, 462-11-1, 462-12-1
PV-ZMBK13+PV-ZMGT09	563-02-1, 576-04-1, 576-04-2, 576-04-5, 576-11-4, 576-18-1, 576-18-3, 576-20-2, 576-22-2, 578-02-1, 579-11-1, 579-15-3, 579-15-4, 581-04-2
PV-ZMBK16	739-09-1, 739-10-1, 742-01-1, 742-01-2
PV-ZMBK16L	747-04-1, 748-01-1, 748-04-3, 754-03-1, 754-04-2, 754-07-4, 754-08-1, 754-10-1, 756-07-1, 756-07-2 , 757-04-1 ,
PV-ZMCT01(PV-ZMBK07+PV-ZMGT10)	475-05-1, 481-10-1, 487-03-1, 487-32-1, 487-46-1, 503-03-1, 512-01-1, 512-03-1, 512-04-1, 513-11-2, 514-02-1, 521-01-1, 523-01-1, 523-04-2, 523-06-1, 523-06-1, 523-07-1, 523-07-2, 523-08-1, 523-09-1, 523-10-1, 530-16-1, 532-06-1, 538-02-1, 540-02-1, 540-02-2, 540-04-1, 540-05-1, 540-07-1, 540-08-1, 544-03-2, 544-04-2, 546-09-1, 546-13-1, 548-08-1, 548-16-3, 549-07-1, 549-10-1, 550-02-1, 550-02-2, 551-02-1, 554-03-1, 554-03-1, 554-03-2, 555-06-1, 557-04-1, 557-04-2, 557-04-4, 557-04-4, 559-08-1, 559-13-2, 559-14-1, 559-14-2, 559-16-1, 559-16-2, 559-31-1, 559-39-1, 559-39-2, 559-41-1, 559-44-1, 559-49-1, 559-50-1, 559-52-1, 559-66-1, 559-80-1, 560-01-1, 562-02-1, 565-12-1, 565-20-2, 569-02-1, 570-01-3, 570-06-1, 570-16-1, 570-22-1, 570-23-2, 570-23-3, 572-03-1, 572-04-1, 572-04-2, 572-05-1, 572-07-1, 572-13-2, 572-16-1, 572-17-1, 572-18-1, 572-24-1, 572-24-1, 572-24-5, 572-24-6, 572-28-1, 574-02-1, 574-04-1, 574-04-2, 574-04-3, 575-03-1, 575-07-1, 575-07-2, 575-07-3, 575-07-4, 575-26-1, 575-26-2, 575-30-1, 575-30-2, 575-31-1, 575-31-2, 575-32-1, 575-32-2, 576-01-1, 576-01-10, 576-01-2, 576-01-4, 576-12-1, 576-12-1, 576-19-1, 578-05-2, 578-05-2, 579-12-1, 581-07-1, 583-03-1, 583-10-1, 583-11-1, 583-11-2, 583-18-1, 584-01-1, 584-02-2, 584-02-3, 584-04-1, 585-01-1, 588-13-1, 588-20-1, 591-01-1, 591-03-1, 591-03-2, 637-11-2, 639-02-1, 639-05-1, 639-08-1, 639-11-1, 645-01-1, 647-03-1, 647-03-2, 647-04-1, 647-07-2, 647-07-4, 647-07-5, 647-08-1, 647-09-1, 647-10-2, 647-10-3, 654-04-1, 657-22-1, 658-01-1, 658-06-1, 658-07-2, 658-11-1, 658-11-2, 661-01-1, 676-17-1, 703-02-1, 714-02-1

TABLE 3. Lines grown at Jerseyville, Illinois (cont.)

Vector	Lines
PV-ZMCT02(PV-ZMBK15+PV-ZMGT03)	568-05-1, 599-01-2, 599-01-3, 599-03-1, 599-04-1, 599-04-2, 599-04-2, 599-04-3, 600-01-1, 600-01-2, 600-08-1, 600-08-2, 600-08-3, 600-08-4, 600-12-1, 600-12-3, 600-12-4, 600-13-1, 600-14-1, 600-14-2, 600-14-3, 600-15-1, 600-15-2, 600-16-1, 600-17-1, 604-03-1, 604-04-1, 604-05-1, 604-05-2, 604-06-1, 604-06-2, 604-09-1, 604-09-1, 604-09-2, 604-09-3, 604-18-1, 604-19-1, 604-24-1, 609-07-2, 610-02-1, 610-07-1, 611-01-1, 611-04-2, 615-07-1, 617-64-1, 617-69-1, 618-40-1, 618-41-1, 618-45-1, 618-46-1, 618-47-2, 618-51-1, 619-10-1, 627-03-1, 627-08-1, 627-08-3, 633-07-1, 634-19-1, 634-22-2, 635-01-1, 635-04-1, 635-06-1, 635-11-1, 635-11-2, 662-06-1, 662-08-1, 666-03-1, 666-06-1, 667-05-1, 671-01-1, 678-06-1, 678-06-1, 680-04-1, 681-05-1
PV-ZMCT04(PV-ZMGT13+PV-ZMGT05)	419-01-1, 425-01-1, 425-01-2, 425-01-3, 425-02-1
PV-ZMCT05(PV-ZMBK13+PV-ZMGT05)	446-18-1, 455-04-1, 462-01-1, 462-03-2, 462-03-3, 462-04-1
PV-ZMCT06(PV-ZMBK17+PV-ZMGT01)	716-07-1, 716-10-1, 730-02-1, 730-02-2, 730-02-3, 733-06-1, 741-09-2, 741-10-1, 745-06-1, 745-06-4, 746-03-1, 747-02-1, 750-01-1, 750-01-2, 750-01-3, 750-01-4, 751-13-1, 751-13-2, 752-06-1, 752-09-1, 756-04-1, 760-11-1, 761-12-2, 762-03-1, 762-12-1
PV-ZMCT07(PV-ZMGT03+PV-ZMGT05)	366-04-1, 370-09-1, 400-01-1, 400-04-2, 400-06-1, 402-08-1, 402-09-1, 423-06-1
PV-ZMCT08(PV-ZMBK07+PV-ZMGT01)	689-09-2, 692-07-1, 694-02-1, 694-06-1, 709-01-1, 709-07-1, 732-11-1, 734-01-1, 734-05-1, 740-03-1, 746-05-1, 767-07-1, 767-09-1, 769-06-1, 769-10-2, 771-07-1, 771-13-1, 779-10-1, 781-05-1, 781-08-1, 783-01-1, 784-02-3, 784-05-1, 785-09-1
PV-ZMCT09(PV-ZMBK25+PV-ZMGT01)	768-06-1, 771-04-2
PV-ZMCT10(PV-ZMBK23+PV-ZMGT10)	766-07-1
PV-ZMCT17(PV-ZMGT03+PV-ZMGT01)	788-03-1
PV-ZMCT33(PV-ZMHS01+PV-ZMGT10)	876-01-1, 876-01-6
PV-ZMCT34(PV-ZMHS02+PV-ZMGT10)	876-04-2
PV-ZMCT38(PV-ZMHS04+PV-ZMGT10)	682-10-1
PV-ZMGT02+PV-ZMGT03	292-05-1, 361-06-1, 365-02-1
PV-ZMGT02+PV-ZMGT04	356-03-1
PV-ZMGT03+PV-ZMGT09	400-05-1
PV-ZMGT04+PV-ZMGT01	347-03-1

TRANSGENIC PLANT ACREAGE AND PLANTING DATES BY LOCATION

Monmouth: Total of approximately 0.7 acres planted 5/14/93 (Regulatory and Bt), 5/15/93 (Roundup)

Jerseyville: Total of approximately 3.5 acres planted 5/17/93 (Regulatory), 6/2/93 and 6/8/93 (all others)

Lockbourne: Total of approximately 0.3 acres planted 5/28/93

RESULTS BY OBJECTIVE

Generate plant material and seed for regulatory submissions
Lines 576-01-1 and 523-06-1 were planted for plant tissue and seed production to support registration. Adequate tissue and seed was produced to meet analytical needs.

Assess weed control efficacy using post-emergence applications of Roundup®
An F1 between lines 356-03-1 and 347-01-1 was used to evaluate weed control. At a given rate and timing, weed control was typically better with crop canopy than in fallow situations.

Assess efficacy of *in-planta* Bt in controlling artificial infestations of European Corn Borer (ECB), and determine genetic segregation
Various generations of Bt lines listed in Tables 2 and 3 (total of approximately 150. lines) were examined for efficacy against first and second generation ECB. 78 lines segregated as expected for a single dominant gene and had ECB scores of 0 or 1 on a 0-9 scale, 0 = no feeding and 9 = severe feeding. These 78 lines were scored for second generation ECB damage using inches of stalk tunnels. Non-Bt lines averaged 18" of tunnels, whereas 69/78 lines averaged 4" or less of tunnels.

Assess efficacy to post-emergence applications of Roundup® of lines genetically modified with genes conferring tolerance to glyphosate, and determine genetic segregation
Various generations of approximately lines listed in Tables 2 and 3 (total of approximately 313 lines) were examined for efficacy against 8 oz/acre, 32 oz/acre, and/or 64 oz/acre of Roundup®. Tolerance varied by line and rate from complete susceptibility at the lowest rate to no distinguishable damage at the highest rate. Approximatley 50% of lines segregated as expected for a single dominant gene.

Produce seed for further testing and evaluation

Harvest from the regulatory trial in Jerseyville and Monmouth was 9/28/93 and 10/20-21/93, respectively. Other trials were harvested from Jerseyville and Monmouth on 9/14-20/93 and 9/20/93, respectively. The plots at Lockbourne, Ohio were destroyed prior to flowering on 8/12/93 by mowing and disking. Tables 4 and 5 detail lines harvested at Monmouth and Jerseyville, respectively.

TABLE 4. Lines harvested from Monmouth, Illinois

Vector	Lines
PV-ZMBK10	631-03-1, 666-08-1, 714-05-1
PV-ZMBK13+PV-ZMGT08	455-11-2
PV-ZMBK13+PV-ZMGT09	578-02-1, 579-11-1
PV-ZMCT01(PV-ZMBK07+PV-ZMGT10)	475-05-1, 481-10-1, 503-03-1, 512-03-1, 523-06-1, 523-09-1, 540-02-2, 540-08-1, 544-03-2, 546-09-1, 554-03-1, 554-03-2, 557-04-1, 557-04-4, 559-16-1, 559-39-2, 559-44-1, 559-52-1, 559-66-1, 560-01-1, 569-02-1, 570-22-1, 572-04-2, 572-16-1, 572-24-1, 574-02-1, 574-04-2, 575-07-2, 575-31-2, 576-01-1, 576-01-10, 578-05-2, 579-12-1, 584-02-2, 591-01-1, 591-03-2, 647-04-1, 654-04-1, 657-22-1, 658-06-1
PV-ZMCT02(PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 600-01-2, 600-13-1, 600-14-2, 600-15-1, 604-09-1, 618-40-1, 618-46-1, 618-47-2, 627-08-1, 635-11-1, 635-11-2, 662-08-1, 678-06-1
PV-ZMCT04(PV-ZMGT13+PV-ZMGT05)	425-01-2, 425-02-1
PV-ZMCT06(PV-ZMBK17+PV-ZMGT01)	756-04-1
PV-ZMCT07(PV-ZMGT03+PV-ZMGT05)	402-08-1, 402-09-1
PV-ZMCT08(PV-ZMBK07+PV-ZMGT01)	689-09-2, 694-02-1, 694-06-1

TABLE 4. Lines harvested from Jerseyville, Illinois

Vector	Lines
PV-ZMBK03+PV-ZMGT01	347-01-1
PV-ZMBK10	631-03-1, 634-07-1, 634-11-1, 666-08-1, 714-05-1
PV-ZMBK10L	749-08-2
PV-ZMBK12	727-04-1
PV-ZMBK12L	749-01-1
PV-ZMBK13+PV-ZMGT08	455-09-3, 455-11-2, 462-05-4, 462-12-1
PV-ZMBK13+PV-ZMGT09	576-11-4, 576-22-2, 578-02-1, 579-11-1, 579-15-3, 579-15-4, 581-04-2
PV-ZMBK16	739-09-1, 742-01-1, 742-01-2
PV-ZMBK16L	748-04-3, 754-03-1, 754-04-2, 754-07-4, 754-08-1, 754-10-1, 756-07-2
PV-ZMCT01(PV-ZMBK07+PV-ZMGT10)	475-05-1, 481-10-1, 487-46-1, 503-03-1, 512-01-1, 512-03-1, 513-11-2, 521-01-1, 523-01-1, 523-04-2, 523-06-1, 523-07-1, 523-08-1, 523-09-1, 523-10-1, 530-16-1, 532-06-1, 540-02-1, 540-02-2, 540-04-1, 540-05-1, 540-08-1, 544-03-2, 544-04-2, 546-09-1, 546-13-1, 548-16-3, 549-07-1, 550-02-1, 551-02-1, 554-03-1, 554-03-2, 557-04-1, 557-04-2, 557-04-4, 559-14-1, 559-16-1, 559-16-2, 559-31-1, 559-39-1

TABLE 4. Lines harvested from Jerseyville, Illinois (cont)

Vector	Lines
PV-ZMCT01(PV-ZMBK07+PV-ZMGT10) (cont)	559-39-2, 559-41-1, 559-44-1, 559-52-1, 559-66-1, 559-80-1, 560-01-1, 562-02-1, 565-12-1, 565-20-2, 569-02-1, 570-06-1, 570-16-1, 570-23-2, 572-03-1, 572-04-1, 572-04-2, 572-13-2, 572-16-1, 572-17-1, 572-18-1, 572-24-1, 572-24-5, 574-02-1, 574-04-1, 574-04-2, 574-04-3, 575-03-1, 575-07-1, 575-07-2, 575-07-4, 575-26-2, 575-30-1, 575-31-1, 575-31-2, 576-01-1, 576-01-10, 576-01-4, 578-05-2, 579-12-1, 581-07-1, 583-03-1, 583-11-2, 583-18-1, 584-01-1, 584-02-3, 584-04-1, 588-13-1, 591-01-1, 591-03-1, 591-03-2, 637-11-2, 639-02-1, 639-11-1, 647-03-1, 647-04-1, 647-07-2, 647-07-4, 647-07-5, 647-10-3, 654-04-1, 657-22-1, 658-01-1, 658-06-1, 658-07-2, 661-01-1, 676-17-1
PV-ZMCT02(PV-ZMBK15+PV-ZMGT03)	568-05-1, 599-01-3, 599-03-1, 599-04-1, 599-04-2, 599-04-3, 600-01-1, 600-01-2, 600-08-2, 600-08-3, 600-12-1, 600-13-1, 600-14-1, 600-14-2, 600-14-3, 600-15-1, 600-15-2, 600-16-1, 600-17-1, 604-03-1, 604-05-1, 604-06-1, 604-06-2, 604-09-1, 604-09-2, 604-09-3, 604-19-1, 604-24-1, 609-07-2, 610-02-1, 611-01-1, 615-07-1, 617-64-1, 618-40-1, 618-41-1, 618-45-1, 618-46-1, 618-47-2, 618-51-1, 627-03-1, 627-08-1, 627-08-3, 633-07-1, 634-22-2, 635-01-1, 635-04-1, 635-11-1, 635-11-2, 662-06-1, 662-08-1, 666-06-1, 671-01-1, 678-06-1
PV-ZMCT04(PV-ZMGT13+PV-ZMGT05)	425-01-1, 425-01-2, 425-01-3, 425-02-1
PV-ZMCT05(PV-ZMBK13+PV-ZMGT05)	455-04-1, 462-01-1, 462-03-2, 462-03-3
PV-ZMCT06(PV-ZMBK17+PV-ZMGT01)	716-07-1, 716-10-1, 730-02-1, 730-02-3, 733-06-1, 741-09-2, 741-10-1, 746-03-1, 747-02-1, 750-01-3, 751-13-1, 751-13-2, 756-04-1, 760-11-1, 762-03-1
PV-ZMCT07(PV-ZMGT03+PV-ZMGT05)	370-09-1, 400-01-1, 400-06-1, 402-08-1, 402-09-1, 423-06-1
PV-ZMCT08(PV-ZMBK07+PV-ZMGT01)	689-09-2, 692-07-1, 694-02-1, 694-06-1, 734-01-1, 734-05-1, 740-03-1, 746-05-1, 767-07-1, 769-10-2, 771-07-1, 771-13-1, 781-05-1, 781-08-1, 783-01-1, 784-02-3, 784-05-1, 785-09-1
PV-ZMCT09(PV-ZMBK25+PV-ZMGT01)	768-06-1
PV-ZMCT10(PV-ZMBK23+PV-ZMGT10)	766-07-1
PV-ZMCT17(PV-ZMGT03+PV-ZMGT01)	788-03-1
PV-ZMCT33(PV-ZMHS01+PV-ZMGT10)	876-01-1, 876-01-6
PV-ZMCT34(PV-ZMHS02+PV-ZMGT10)	876-04-2

TABLE 4. Lines harvested from Jerseyville, Illinois (cont)

Vector	Lines
PV-ZMCT38(PV-ZMHS04+PV-ZMGT10)	682-10-1
PV-ZMGT02+PV-ZMGT03	292-05-1, 361-06-1, 365-02-1
PV-ZMGT02+PV-ZMGT04	356-03-1
PV-ZMGT03+PV-ZMGT09	400-05-1

Plots were destroyed within approximately one week of harvest by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines varied with respect to both how much non-recurrent parent (Hi-Type II) was present and the degree of inbreeding. Hi-Type II as a line varies considerably with respect to phenotype. No significant deviation from expected phenotype was observed among the transformed lines.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to nontransgenic plants of similar genotype. Rust and leaf blight were present in both transgenic and non-transgenic plots. Disease levels were similar in both types of materials; rust infection ranged upwards to 80% by season end in some plots. Leaf blight, primarily Southern Leaf Blight (SLB), was more prevalent on smaller, less vigorous plots of Hill material, individual transgenic and non-transgenic plants exhibiting almost 90% infection by season end. More typical infection of rust was in the range of 10-20%, and for SLB, 5-20%. Transgenic plants appeared no more or less susceptible than non-transgenic plants.

Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to nontransgenic plants of similar genotype, except for Bt plants, which appeared more tolerant to natural infestations of ECB, as expected.

Weediness

All transgenic plants exhibited normal corn phenotypes that preclude shattering or other forms of seed dispersal prior to manual harvest. Husks remained over the ears, and seed remained attached to the cob. No evidence of seed dormancy or delayed germination was observed. Corn is unable to propagate vegetatively following maturity, and this characteristic appeared unchanged in these lines.

ISOLATION

All open-pollinated corn within 200 meters of the transgenic plots was destroyed.

VOLUNTEERS

Lockbourne, OH:
Crop was destroyed prior to flowering, and no seed was set that could cause volunteer corn in the following season. No volunteers were noted.

Monmouth, IL:
No volunteer plants were observed following crop destruct. Approximately 160 volunteer corn plants were counted in July, 1994. These were removed prior to flowering.

Jerseyville, IL:
No volunteer plants were observed following crop destruct. No volunteer plants were observed during the April or May observations. Soybeans were planted over the site. In June, approximately 150 plants were counted and removed in July prior to flowering.

SUMMARY REPORT OF FIELD TEST DATA

Approved USDA Ref. Numbers: 93-144-02N
Monsanto Ref. Number: 93-069RA

PURPOSE

Introgress the *B.t.* gene into elite germplasm through several cycles of backcrossing.

GENERAL RESULTS OF EXPERIMENT

This permit covered 2 plantings at the [Pioneer research station at Salinas, Puerto Rico.]

The first planting occurred on September 3, 1993. This planting was .15 transgenic acres and .15 total acres. Line numbers included in this planting were:

PV-ZMCT01 557-04-4, 575-31-2, 588-13-1, 572-24-1, 554-03-2, 654-04-1, 546-09-1, 576-01-1, and 572-16-1.
(PV-ZMBK07 + PV-ZMGT10)

PV-ZMCT02 604-09-1, 600-14-2, 599-04-2, 635-11-2, 600-13-1, and 618-40-1.
(PV-ZMBK15 + PV-ZMGT03)

Each of these lines were backcrossed to their recurrent parent - an elite inbred. Also, four of the lines were self pollinated. The seed was harvested on December 19, 1993. The backcross material was sent to the facility at [Johnston, Iowa].

The second planting occurred on 12/24/93 and included lines:

PV-ZMCT01 572-16-1, 523-06-1, 546-09-1, and 576-01-1.

Each of the lines were crossed to several elite inbreds to make experimental hybrids. The seed was harvested on April 4, 1994 and sent to the facility at [Johnston, Iowa].

POLLEN CONTAINMENT

All open pollinated ears with 660 feet of the transgenic trials were destroyed by disking under.

GENERAL FIELD OBSERVATIONS

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

GENERAL FIELD OBSERVATIONS (Continued)

Observations of the modified plants showed normal fertility and seed set.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease or pest susceptibility.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

FINAL DISPOSITION

All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING

The plots were cultivated then irrigated to promote germination and any resulting volunteers were destroyed. This cycle was repeated until no volunteers emerged. After approximately 60 days the plots were replanted to corn.

SUMMARY REPORT OF FIELD TEST DATA

Approved USDA Ref. Numbers: 93-245-02N
Monsanto Ref. Number: 93-099RA

PURPOSE

Introgress the *B.t.* gene into elite germplasm through several cycles of backcrossing.

GENERAL RESULTS OF EXPERIMENT

This permit was used to cover plantings at the [Pioneer research station at Kekaha Kauai, Hawaii.]

The first planting occurred on November 6, 1993. This planting was 0.1 transgenic acres and 0.2 total acres. Line numbers included in this planting were:

PV-ZMCT01 588-13-1, 572-16-1, 513-11-2, 570-22-1, 544-04-2, 658-06-1, 581-07-1, 576-01-1, and 676-17-1. (PV-ZMBK07 + PV-ZMGT10)

PV-ZMCT02 604-09-1, 599-04-2, 635-11-2, and 600-08-3. (PV-ZMBK15 + PV-ZMGT03)

PV-ZMBK10 714-05-1, 634-11-1, and 631-03-1.

PV-ZMBK12L 749-01-1

PV-ZMBK16L 754-08-1, 754-10-1, and 748-04-3.

Each of these lines were backcrossed to their recurrent parent - an elite inbred. Also, the lines were crossed to an elite inbred to make experimental hybrid seed. The seed was harvested on 2/23/94. A portion of the backcross material was sent to the facility at [Johnston, Iowa] and the remainder was replanted in Hawaii in the next planting. All of the crossing seed was sent to [Johnston,Iowa] for yield testing.

The second planting occurred on 12/2/93 and contained the same lines as the first planting. Material in this planting was treated the same as that in the first planting. Harvest occurred on 3/29/94-4/13/94. Upon harvesting, seed was shipped as identified above.

GENERAL RESULTS OF EXPERIMENT (Continued)

The third, fourth and fifth plantings occurred March 4, 17 and April 11, 1994. Line numbers included in this planting were:

PV-ZMCT01 572-16-1, 513-11-2, 544-04-2, 658-06-1, 581-07-1, 576-01-1, 588-13-1, 572-24-1, and 676-17-1. (PV-ZMBK07 + PV-ZMGT10)

PV-ZMCT02 604-09-1, 599-04-2, 635-11-2, and 600-08-3. (PV-ZMBK15 + PV-ZMGT03)

PV-ZMBK10 714-05-1, 634-11-1, and 631-03-1.

PV-ZMBK12L 749-01-1

PV-ZMBK16L 754-08-1, and 754-10-1.

Material in these plantings was backcrossed to the recurrent parent and self pollinated. The seed was harvested on June 17, 28 and July 8 respectively. A portion of the backcross and self seed was sent to the [Pioneer facility at Johnston, Iowa] and the remainder was replanted in the next planting.

POLLEN CONTAINMENT

All open pollinated ears within 660 feet of the transgenic trials were destroyed by disking under.

GENERAL FIELD OBSERVATIONS

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed normal fertility and seed set.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease or pest susceptibility.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

FINAL DISPOSITION

All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING

The plots were cultivated then irrigated to promote germination and any resulting volunteers were destroyed. This cycle was repeated until no volunteers emerged. After approximately 60 days the plots were replanted to corn.

FINAL REPORT

USDA No. 93-250-04N
Monsanto No. 93-101RA

OBJECTIVE

Self and backcross glyphosate tolerant plants in a breeding nursery .

GENERAL RESULTS OF EXPERIMENT

No field experiments were established under this Notification (93-250-04N) which was granted October 15, 1993.

DISPOSAL, GENERAL FIELD OBSERVATIONS, MONITORING FOR VOLUNTEERS

None as site was not established.

Kent A. Croon
Regulatory Affairs Manager

APHIS# 93-258-04N
MON# 93-121R

HAWAII OFF-SEASON TESTING AND BREEDING NURSERY
NOVEMBER, 1993 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-258-04N
Monsanto: 93-121R

EXPERIMENT DESCRIPTION

The objective of the bulk of the planting was to produce seed for testing during the 1994 mainland U.S. growing season. As part of that production, lines were evaluated for segregation of genes coding for the insecticidal protein and/or tolerance to glyphosate. The objective of a second trial was to determine the difference in yield between four pairs of lines, the pairs differing with respect to the presence or absence of the insect resistance gene. A third trial evaluated the yield of a single line under different application regimes of glyphosate.

Plots were located on Molokai, Hawaii (Maui County). The trials were planted 11/19/93 - 11/24/93 over an area of 2.5 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested 3/14/94 - 3/21/94 based on maturity.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or detasselling of transgenic lines.

RESULTS

Seed production was as expected under Hawaiian conditions. Lines were selected based on normalcy of segregation and tolerance to glyphosate. In the yield experiments, for the single line evaluated, yield decreased and moisture increased with later glyphosate applications. Comparisons of yield between pairs of lines with and without the insect resistance gene showed only one of four lines tested may have had a negative effect from the gene insertion. This effect was manifested by later maturity reflected by higher moisture. Lines showing negative effects from the glyphosate or gene per se have been eliminated from further development.

Vector	Lines
PV-ZMBK10	631-03-1, 634-11-1, 714-05-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-08-1, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 512-03-1, 513-11-2, 523-06-1, 523-09-1, 540-04-1, 544-03-2, 544-04-2, 546-09-1, 559-39-2, 559-52-1, 572-04-2, 572-16-1, 572-24-1, 574-02-1, 574-04-2, 575-07-2, 575-26-2, 575-30-1, 576-01-1, 579-12-1, 581-07-1, 588-13-1, 591-03-2, 639-02-1, 658-06-1, 676-17-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 600-08-3, 604-09-1, 627-08-1, 635-11-1, 635-11-2

GENERAL FIELD OBSERVATIONS

Plots were observed twice during the growing season, before and after flowering. No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. Rust was the primary disease observed. Insect pressure was primarily from corn ear worms. Some insect protected lines appeared to have less ear damage than non-insect protected lines and non-transgenic lines. This will be investigated in future experiments.

DISPOSAL

Remaining plant material after harvest was disked into the soil 3/18/94 - 4/4/94.

VOLUNTEERS

Following cultivation, the fields were irrigated, seed allowed to germinate, and resulting volunteer plants destroyed by mechanical or hand cultivation. This cycle was repeated four times, from immediately after initial cultivation through 7/8/94. Volunteers were estimated after the initial irrigation cycle to be approximately 2 plants per square foot, while after the last irrigation, only about 2 plants per acre were found. While the initial plant density is a function of the amount of unharvested ears left in the field prior to plot destruction, the occurrence of volunteers in this trial and their subsequent elimination is very typical of that seen historically with non-transgenic plantings.

Vector	Lines
PV-ZMBK10	631-03-1, 634-11-1, 714-05-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-08-1, 754-10-1
PV-ZMCT01	481-10-1, 512-03-1, 513-11-2, 523-06-1, 523-09-1,
(PV-ZMBK07+PV-ZMGT10)	540-04-1, 544-03-2, 544-04-2, 546-09-1, 559-39-2,
	559-52-1, 572-04-2, 572-16-1, 572-24-1, 574-02-1,
	574-04-2, 575-07-2, 575-26-2, 575-30-1, 576-01-1,
	579-12-1, 581-07-1, 588-13-1, 591-03-2, 639-02-1,
	658-06-1, 676-17-1
PV-ZMCT02	599-04-2, 599-04-3, 600-08-3, 604-09-1 627-08-1,
(PV-ZMBK15+PV-ZMGT03)	635-11-1, 635-11-2
PV-ZMCT04	425-01-2, 425-02-1
(PV-ZMGT13+PV-ZMGT05)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	751-13-1, 762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT08	694-02-1, 694-06-1, 767-07-1
(PV-ZMBK07+PV-ZMGT01)	
PV-ZMCT09	768-06-1
(PV-ZMBK23+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

GENERAL FIELD OBSERVATIONS

No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. Transgenic plants did not demonstrate any evidence of the potential for increased weediness.

DISPOSAL

Remaining plant material after harvest was disked into the soil after harvest.

VOLUNTEERS

Recurring cycles of irrigation and cultivation elliminated volunteer plants.

[Ciba Seeds], Molokai (Maui County), HI]
OFF-SEASON TESTING AND BREEDING NURSERY
MAY, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-279-04N phenotype: Lepidopteran insect resistance
93-281-02N phenotype: Glyphosate tolerant
93-281-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 93-135R
93-136R
93-137R

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress the genes of interest into proprietary inbred lines.

The trial was planted 5/23/94 over an area of 0.125 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector. Plots were harvested at maturity.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or bagging of tassels shedding pollen.

RESULTS

Seed production was as expected under Hawaiian conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 513-11-2, 540-04-1, 544-03-2, 557-04-4,
(PV-ZMBK07+PV-ZMGT10)	559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2,
	576-01-1, 581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

GENERAL FIELD OBSERVATIONS

No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. Transgenic plants did not demonstrate any evidence of the potential for increased weediness.

DISPOSAL

Remaining plant material after harvest was disked into the soil after harvest.

VOLUNTEERS

Recurring cycles of irrigation and cultivation eliminated volunteer plants.

[Ciba Seeds], Molokai (Maui County), HI
OFF-SEASON TESTING AND BREEDING NURSERY
AUGUST, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-279-04N phenotype: Lepidopteran insect resistance
93-281-02N phenotype: Glyphosate tolerant
93-281-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 93-135R
93-136R
93-137R

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress the genes of interest into proprietary inbred lines.

The trial was planted 8/11/94 over an area of 0.168 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector. Plots were harvested at maturity.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or bagging of tassels shedding pollen.

RESULTS

Seed production was as expected under Hawaiian conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 540-04-1, 544-03-2, 554-03-2, 557-04-4,
(PV-ZMBK07+PV-ZMGT10)	559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2,
	576-01-1, 581-07-1, 591-03-2, 639-02-1, 654-04-1,
	658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 600-14-2, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT07	423-06-1
(PV-ZMGT03+PV-ZMGT05	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

GENERAL FIELD OBSERVATIONS

No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. Transgenic plants did not demonstrate any evidence of the potential for increased weediness.

DISPOSAL

Remaining plant material after harvest was disked into the soil after harvest.

VOLUNTEERS

Recurring cycles of irrigation and cultivation eliminated volunteer plants.

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HAWAII OFF-SEASON TESTING AND BREEDING NURSERY

FINAL REPORT

Kent A. Croon
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-308-02N
Monsanto: 93-156R

EXPERIMENT DESCRIPTION

The objective of this trial was the screening and propagation of various selections of genetically modified corn inbreds for resistance to Roundup® (glyphosate) and to lepidopteran insects. Information in this report was provided by [CBI DELETED] [CBI DELETED]

RESULTS

The trial was planted on three dates, December 6th, 8th, and 10th of 1994, at [CBI DELETED] [CBI DELETED] on the island of Molokai in the state of Hawaii. The specific field sites were in Hawaiian Research's fields 18A-3 and 18A-4 and had a combined acreage of 0.45 acres of which 0.07 acres were genetically modified material. The plots were isolated from the other non-transgenic corn by a minimum of 660' and/or by over one month by planting dates in accordance with seed industry standards to insure no viable transgenic corn pollen contaminated any non-transgenic corn plants. The genetically modified lines included in this trial are listed on the following page.

At maturity of the plot, harvest of selected plants began. Harvest began on March 28, 1994 and was completed on March 30, 1994. The final result of the trial was the advancement of the genetics of the screened plants through crossing and self pollination.

Vector	Lines
PV-ZMBK10	631-03-1, 634-11-1, 714-05-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-08-1, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 512-03-1, 513-11-2, 523-06-1, 523-09-1, 540-04-1, 544-03-2, 544-04-2, 546-09-1, 559-39-2, 559-52-1, 572-04-2, 572-16-1, 572-24-1, 574-02-1, 574-04-2, 575-07-2, 575-26-2, 575-30-1, 576-01-1, 579-12-1, 581-07-1, 588-13-1, 591-03-2, 639-02-1, 658-06-1, 676-17-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 600-08-3, 604-09-1, 627-08-1, 635-11-1, 635-11-2

GENERAL FIELD OBSERVATIONS

During the course of this trial, observations were made as to any apparent differences between the genetically modified corn lines and non-modified lines within the plot. No noticeable differences could be detected between the transgenic and non-transgenic corn plants. The plot showed the normal pressures expected from leaf blights and nontarget insects throughout the field with no differentiation between normal and modified plants. Germination, plant growth, and phenotypic characteristics were normal throughout the plot.

DISPOSAL

The remaining corn and dry plant material were then mowed, disced, and irrigated to begin germination of all discarded and contaminated seed.

VOLUNTEERS

The plot area was monitored for volunteer plants and then disced to destroy any germinating seed. This cycle of discing-irrigation-monitoring was continued until there were no volunteer plants noted. The site was monitored monthly for a four month period for volunteers and corresponding numbers of plants noted; month one: 2-22 plants/sq. ft., month two: 1 plant/sq. ft., month three: 2-5 plants/Acre, month four: 1-2 plants per acre.

[ASGROW Seed Company]
[Isabela, Puerto Rico]
OFF-SEASON TESTING AND BREEDING NURSERY
February, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-306-04N phenotype: Lepidopteran insect resistance
93-306-06N phenotype: Lepidopteran insect resistant/Glyphosate tolerant
Glyphosate tolerant

Monsanto: 93-152R
93-153R

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress genes of interest into proprietary inbred lines. Lepidopteran insect resistant and glyphosate tolerant lines were contained within the same breeding nursery. Therefore, this final report is prepared as a summary of the two USDA Notifications identified above.

The trials were planted February 10, 1994, over an area of 0.3 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested May 19, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or detasselling of transgenic lines.

RESULTS

Seed production was as expected under Puerto Rico conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	754-07-4, 754-10-1, 748-04-3
PV-ZMCT01 (PV-ZMBK07+ PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-04-2, 554-03-2, 557-04-4, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 654-04-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+ PV-ZMGT03)	599-04-2, 599-04-3, 600-14-2
PV-ZMCT05 (PV-ZMBK13+ PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+ PV-ZMGT01)	762-03-1
PV-ZMCT07 (PV-ZMGT03+ PV-ZMGT05)	423-06-1
PV-ZMCT10 (PV-ZMBK23+ PV-ZMGT10)	766-07-1

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic and proprietary inbred lines. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents.

DISPOSAL

Remaining plant material after harvest was disked into the soil June 2, 1994.

VOLUNTEERS

Volunteers were monitored and destroyed as they appeared.

[ASGROW Seed Company]
[Isabela, Puerto Rico]
OFF-SEASON TESTING AND BREEDING NURSERY
June, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-306-04N phenotype: Lepidopteran insect resistance
93-306-06N phenotype: Lepidopteran insect resistance/Glyphosate tolerant
Glyphosate tolerant

Monsanto: 93-152R
93-153R

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress genes of interest into proprietary inbred lines. Lepidopteran insect resistant and glyphosate tolerant lines were contained within the same breeding nursery. Therefore, this final report is prepared as a summary of the two USDA Notifications identified above.

The trials were planted June 23, 1994, over an area of 0.35 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested September 1, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or detasselling of transgenic lines.

RESULTS

Seed production was as expected under Puerto Rico conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 513-11-2, 540-04-1, 544-04-2, 559-39-2,
(PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1, 574-04-2, 575-07-2, 581-07-1, 591-03-2
PV-ZMCT02	599-04-2, 599-04-3
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic and proprietary inbred lines. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. In plots containing *Bt* lines, insect feeding was less pronounced than in non-transgenic plots. In transgenic plots without the *Bt* gene, no disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents.

DISPOSAL

Remaining plant material after harvest was disked into the soil September 26, 1994.

VOLUNTEERS

Volunteers were monitored and destroyed as they appeared.

[ASGROW Seed Company]
[Isabela, Puerto Rico]
OFF-SEASON TESTING AND BREEDING NURSERY
July, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA:	93-306-04N	phenotype:	Lepidopteran insect resistance
	93-306-06N	phenotype:	Lepidopteran insect resistant/Glyphosate tolerant Glyphosate tolerant
Monsanto:	93-152R		
	93-153R		

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress genes of interest into proprietary inbred lines. Lepidopteran insect resistant and glyphosate tolerant lines were contained within the same breeding nursery. Therefore, this final report is prepared as a summary of the two USDA Notifications identified above.

The trials were planted July 5, 1994, over an area of 0.3 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested September 9, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or detasselling of transgenic lines.

RESULTS

Seed production was as expected under Puerto Rico conditions.

Vector	Lines
PV-ZMBK16L	748-04-3
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	554-03-2, 557-04-4, 576-01-1, 654-04-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	600-14-2
PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	423-06-1

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic and proprietary inbred lines. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents.

DISPOSAL

Remaining plant material after harvest was disked into the soil following harvest.

VOLUNTEERS

Volunteers were monitored and destroyed as they appeared.

[HOLDEN'S FOUNDATION SEED]
[Molokai (Maui County), Hawaii]
OFF-SEASON TESTING AND BREEDING NURSERY
January, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-316-04N phenotype: Lepidopteran insect resistance
93-316-06N phenotype: Lepidopteran insect resistant/Glyphosate tolerant
93-316-08N phenotype: Glyphosate tolerant

Monsanto: 93-158R
93-159R
93-160R

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress genes of interest into proprietary inbred lines. The lines identified below were planted into the same winter nursery block and included all three phenotypes identified above.

The trials were planted January 4, 1994, over an area of 0.09 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested May 1, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or detasselling of transgenic lines.

RESULTS

Seed production was as expected under Hawaii conditions.

Vector	Lines
PV-ZMBK10	631-03-1, 634-11-1, 714-05-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-08-1, 754-10-1
PV-ZMCT01	481-10-1, 512-03-1, 513-11-2, 523-09-1, 540-04-1,
(PV-ZMBK07+PV-ZMGT10)	544-03-2, 544-04-2, 559-39-2, 559-52-1, 572-04-2,
	572-16-1, 572-24-1, 574-02-1, 574-04-2, 575-07-2,
	575-26-2, 575-30-1, 576-01-1, 579-12-1, 581-07-1,
	588-13-1, 591-03-2, 639-02-1, 658-06-1, 676-17-1
PV-ZMCT02	599-04-2, 599-04-3, 600-08-3, 604-09-1, 627-08-1,
(PV-ZMBK15+PV-ZMGT03)	635-11-1, 635-11-2
PV-ZMCT04	425-01-2, 425-02-1
(PV-ZMGT13+PV-ZMGT05)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	751-13-1, 762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT08	694-02-1, 694-06-1, 767-07-1
(PV-ZMBK07+PV-ZMGT01)	
PV-ZMCT09	768-06-1
(PV-ZMBK23+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic and proprietary inbred lines. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents.

DISPOSAL

Remaining plant material after harvest was disked into the soil after harvest.

VOLUNTEERS

After harvest and initial disking of plant residues, three cycles of irrigation-2 weeks fallow-disking was used for volunteer control. Number of volunteer corn plants decreased with each successive cycle until no volunteer plants were observed.

[HOLDEN'S FOUNDATION SEED]
[Molokai (Maui County), Hawaii]
OFF-SEASON TESTING AND BREEDING NURSERY
May, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-316-04N phenotype: Lepidopteran insect resistance
93-316-06N phenotype: Lepidopteran insect resistant/Glyphosate tolerant
93-316-08N phenotype: Glyphosate tolerant

Monsanto: 93-158R
93-159R
93-160R

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress genes of interest into proprietary inbred lines. The lines identified below were planted into the same winter nursery block and included all three phenotypes identified above.

The trials were planted May 13, 1994, over an area of 0.15 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested August 15, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or detasselling of transgenic lines.

RESULTS

Seed production was as expected under Hawaii conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-03-2, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 604-09-1
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	762-03-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic and proprietary inbred lines. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents.

DISPOSAL

Remaining plant material after harvest was disked into the soil after harvest.

VOLUNTEERS

After harvest and initial disking of plant residues, three cycles of irrigation-2 weeks fallow-disking was used for volunteer control. Number of volunteer corn plants decreased with each successive cycle until no volunteer plants were observed.

[HOLDEN'S FOUNDATION SEED]
[Molokai (Maui County), Hawaii]
OFF-SEASON TESTING AND BREEDING NURSERY
September, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-316-04N phenotype: Lepidopteran insect resistance
93-316-06N phenotype: Lepidopteran insect resistant/Glyphosate tolerant
93-316-08N phenotype: Glyphosate tolerant

Monsanto: 93-158R
93-159R
93-160R

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress genes of interest into proprietary inbred lines. The lines identified below were planted into the same winter nursery block and included all three phenotypes identified above.

The trials were planted September 1, 1994, over an area of 0.43 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested December 15, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial or detasselling of transgenic lines.

RESULTS

Seed production was as expected under Hawaii conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 559-39-2, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	762-03-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic and proprietary inbred lines. Nothing unusual was noted as transgenic plants were compared to non-transgenic plants. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents.

DISPOSAL

Remaining plant material after harvest was disked into the soil after harvest.

VOLUNTEERS

After harvest and initial disking of plant residues, three cycles of irrigation-2 weeks fallow-disking was used for volunteer control. Number of volunteer corn plants decreased with each successive cycle until no volunteer plants were observed.

HAWAII OFF-SEASON TESTING AND BREEDING NURSERY

FINAL REPORT

Kent A. Croon
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-354-06N
Monsanto: 93-179R

EXPERIMENT DESCRIPTION

The objective of the trial was to self and backcross plants expressing insect tolerance in a breeding nursery.

Plot location: [ICI Seeds Research Station, 94-880 Kunia Road, Honolulu County, Kunia].

Trial Acreage: 0.04 acres genetically modified.
0.06 total acres

Planting date: January 19, 1994

Harvest date: May 2, 1994

Isolation method: Bagging of tassels of transgenic plants.

RESULTS

Laboratory assays were performed on leaf tissue from individual plants in order to identify those plants expressing the insect resistance gene at suitable levels. Insect protected plants were selfed and backcrossed to the elite recipient inbred. Ears from selfed and backcrossed plants were hand-harvested and returned to [the ICI Seeds Research Center, Slater,] IA.

Vector	Lines
PV-ZMBK10	631-03-1, 634-11-1, 714-05-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-08-1, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	513-11-2, 523-06-1, 544-04-2, 572-16-1, 572-24-1, 576-01-1, 581-07-1, 588-13-1, 639-02-1,658-06-1, 676-17-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 600-08-3, 604-09-1, 635-11-2

GENERAL FIELD OBSERVATIONS

The transgenic plants were not abnormally susceptible to disease or insects. The transgenic plants did not exhibit abnormal germination, tasseling, or seed production that would confer a weediness trait. The plant growth and morphology of transgenic plants was similar to that of non-transgenic plants.

DISPOSAL

The remaining plant material, including unharvested ears, was disked and plowed into the field.

VOLUNTEERS

Following the disking and plowing, the field plot was irrigated and monitored for volunteer corn plants. No volunteers were observed over the next 30 days.

HAWAII OFF-SEASON TESTING AND BREEDING NURSERY
MARCH, 1994 PLANTING

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-026-04N phenotype: Lepidopteran insect resistance
94-026-05N phenotype: Lepidopteran insect resistant/Glyphosate tolerant
94-026-06N phenotype: Glyphosate tolerant

Monsanto: 94-023XRA
94-024XRA
94-026XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to produce seed for testing and to introgress the genes of interest (GOI) into elite inbred backgrounds. All three phenotypes identified above were planted into the same nursery block within the same isolation distance.

Plots were located on Molokai, Hawaii (Maui County). The trial was planted 3/22/94 over an area of 0.2 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested 7/8/94, based on maturity.

The isolation method utilized was at least 200 meter isolation from open-pollinated corn not to be destroyed.

RESULTS

Seed production was as expected under Hawaiian conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-03-2, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 604-09-1
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	762-03-1
PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	423-06-1
V-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

GENERAL FIELD OBSERVATIONS

No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. Rust was the primary disease observed. Insect pressure was primarily from corn ear worms. Some *Bt* lines appeared to have less ear damage than non-Bt lines and non-transgenic lines. No characteristics that may lead to increased weediness were noted.

DISPOSAL

Remaining plant material after harvest was disked into the soil.

VOLUNTEERS

Following cultivation, the fields were irrigated, seed allowed to germinate, and resulting volunteer plants destroyed by mechanical or hand cultivation. This cycle was repeated until no volunteer plants were observed.

PUERTO RICO OFF-SEASON TESTING AND BREEDING NURSERY
APRIL, 1994 PLANTING

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-026-04N phenotype: Lepidopteran insect resistance
94-026-05N phenotype: Lepidopteran insect resistant/Glyphosate tolerant
94-026-06N phenotype: Glyphosate tolerant

Monsanto [redacted]
94-024XRA
94-026XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to produce seed for testing and to introgress the genes of interest (GOI) into elite inbred backgrounds. All three phenotypes identified above were planted into the same nursery block within the same isolation identified below.

The trial was established at the Monsanto Research Farm in Santa Isabel, Puerto Rico, near Ponce. The trial was planted 4/12/94 over an area of 0.05 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested at maturity.

The isolation method utilized was at least 200 meter isolation from open-pollinated corn not to be destroyed.

RESULTS

Seed production was as expected under Puerto Rico conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 513-11-2, 540-04-1, 544-03-2, 559-39-2,
(PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1,
	581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT07	423-06-1
(PV-ZMGT03+PV-ZMGT05)	
V-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

GENERAL FIELD OBSERVATIONS

No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. No characteristics that may lead to increased weediness were noted.

DISPOSAL

Remaining plant material after harvest was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at harvest. Irrigation was applied to the plot area with tillage used to remove volunteers. This process was repeated until no volunteers were evident.

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1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
The Agricultural Group of Monsanto

Cooperator Name and Address	Site Location
[CBI DELETED]	Wayne County, North Carolina

Permit Numbers 94-011XRA
94-024-02N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/07/94 **Harvest Date** 09/01/94

Trial Size 0.26 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Incorporated in soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

10/02/94	>10,000	04/02/95	0
1/01/94	0	07/05/95	0

Method used to Destroy Volunteers Disking

Other comments

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-024-03N
Pioneer Number:	CORN-IA-94-02
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Telephone Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE

Yield determination, gene efficacy, and seed production.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered eight planting sites at Center Point, Linn County; Vinton, Benton County; Algona, Kossuth County; Callendar, Webster County; Johnston, Polk County; Sheldahl, Polk County; Melbourne, Marshall County; Scranton, Greene County, Iowa.

Site by County	Plant Date	Acreage	Number of Plants	Evaluation Purpose	Harvest Date
Benton	5/17/94	0.21	4,100	yield determination	10/14/94
Greene	5/16/94	0.60	12,000	yield determination	10/8/94
Kossuth	5/10/93	0.23	4,600	yield determination and gene efficacy	10/10/94
Linn	5/17/94	0.38	7,500	yield determination and gene efficacy	10/13/94
Marshall	5/17/94	0.58	11,500	yield determination	10/8/94
Polk	5/18/94 and 5/19/94	2.78	55,600	yield determination, gene efficacy, and seed production	10/8/94
Webster	5/10/94	0.23	4,600	yield determination	10/10/94

The trials were isolated by a distance of at least 660 feet from any other corn. The grain from all rows was harvested by machine on the dates noted above. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growingseason. Observations of the plants were recorded at different stages during the trials, and the results are listed below.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-024-04N
Pioneer Number:	CORN-IL-94-03
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Telephone Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE
Yield determination, efficacy, seed production.

This permit covered five planting sites at Seymour, Macomb, Dover, Shelbyville, and Long Point, Illinois

Site	Plant Date	Acreage	Number of Plants	Evaluation Purpose	Harvest Date
Seymour	5/20/94	0.30	6,000	yield determination	10/14/94
Macomb	5/10/94	0.30	6,000	yield determiantion	10/19/94
Dover	5/18/94	0.90	18,000	yield determination and gene efficacy	10/26/94
Shelbyville	5/20/94	0.28	5,640	yield determination	10/6/94
Long Point	5/17/94	0.33	6,600	yield determination	10/20/94

The trials were isolated by a distance of at least 660 feet from any other corn. The grain from all rows was harvested by machine on the dates noted above. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growingseason. Observations of the plants were recorded at different stages during the trials, and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not discloseany deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was chopped and returned to the plot for soil composting.

ILBT04.DOC

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-024-05N
Pioneer Number:	CORN-SD-94-04
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer
	11252 Aurora Avenue
	Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE

Yield determination and gene efficacy.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered one planting site at our research/breeding station located in Huron, Beadle County, South Dakota. The trial was planted on May 13, 1994, and comprised 0.7 acres. The trial was isolated by a distance of at least 660 feet from any other corn. Approximately 14,000 plants were evaluated for yield determination and for insect resistance. The grain from all rows was harvested by machine on October 13, 1994. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At two different stages the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not discloseany deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human healtheffects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was chopped and returned to the plot for soil composting.

SUMMARY REPORT OF FIELD TEST DATA

Approved USDA Number:	94-024-06N
State of Wisconsin Number:	94-12
Pioneer Number:	CORN-WI-94-05
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer
	11252 Aurora Avenue
	Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE
Yield determination.

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site at our research/breeding station located in Janesville, Rock County, Wisconsin.

The site was planted on May 13, 1994 and consisted of approximately 0.26 acre of transgenic corn. Approximately 5,500 plants were evaluated for yield determination. The trial was isolated by a distance of at least 660 feet from any other corn. The grain from all rows was harvested by machine on October 27, 1994. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At four different stages the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not discloseany deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

SUMMARY REPORT OF FIELD TEST DATA

Approved USDA Number:	94-024-07N
Pioneer Number:	CORN-MI-94-06
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer
	11252 Aurora Avenue
	Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE

Yield determination.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered one planting site at our research/breeding station located in Breckenridge, Gratiot County, Michigan. The trial was planted on May 13, 1994 and consisted of 0.18 acre. The trial was isolated by a distance of at least 660 feet from any other corn. Approximately 3,600 transgenic plants were evaluated for yield determination. The grain from all rows was harvested by machine on October 27, 1994. The grain was spread on the ground at the trial site, then tilled into the soil along with the remaining vegetative material. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. Observation of the trial was recorded on July 7, 1994 and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was chopped and returned to the plot for soil composting.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-024-08N
Pioneer Number:	CORN-MN-94-07
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer
	11252 Aurora Avenue
	Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Telephone Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE

Yield determination.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered one planting site at our research/breeding station located in Mankato, Blue Earth County, Minnesota. The trial was planted on May 9, 1994, and comprised 0.18 acre. The trial was isolated by a distance of at least 660 feet from any other corn. Approximately 3,500 transgenic plants were evaluated for yield determination. The grain from all rows was harvested by machine on October 11, 1994. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At two different stages the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not discloseany deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was chopped and returned to the plot for soil composting.

SUMMARY REPORT OF FIELD TEST DATA

Approved USDA Number:	94-024-09N
Pioneer Number:	CORN-PA-94-08
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer
	11252 Aurora Avenue
	Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE
Yield determination.

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site at our research/breeding station located in Lancaster, Lancaster County, Pennsylvania.

The site was planted on May 19, 1994 and consisted of approximately 0.27 acre of transgenic corn. Approximately 5,400 plants were evaluated for yield determination. The trial was isolated by a distance of at least 660 feet from any other corn. The grain from all rows was harvested by machine on October 13, 1994. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At four different stages the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and return to the plot for soil composting.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-024-10N
Pioneer Number:	CORN-IN-94-09N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Telephone Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE

Yield determination, gene efficacy, and seed production.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered three planting sites at Wheatfield, Jasper County; Tipton, Tipton County; and Priceton, Gibson County, Indiana. The Windfall, Tipton County, Indiana, site was not planted.

Site by County	Plant Date	Acreage	Number of Plants	Evaluation Purpose	Harvest Date
Jasper	5/20/94	0.33	6,500	yield determination	10/17/94
Tipton	5/5/94 and 5/20/94	0.53	10,500	yield determination and gene efficacy	10/14/94
Gibson	5/19/94	0.05	900	yield determination	10/19/94

The trials were isolated by a distance of at least 660 feet from any other corn. The grain from all rows was harvested by machine on the dates noted above. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growingseason. Observations of the plants were recorded at different stages during the trials, and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodifiedcontrols.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not discloseany deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was chopped and returnedto the plot for soil composting.

SUMMARY REPORT OF FIELD TEST DATA

Approved USDA Number:	94-024-11N
Pioneer Number:	CORN-NE-94-10
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer
	11252 Aurora Avenue
	Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE

Yield determination, gene efficacy, and seed production.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered two planting sites near our research/breeding station located in York, York County, Nebraska. The original application specified 0.5 acres for each site, however, by a May 3, 1994 letter we corrected the area to 1.5 acres for the first site.

The first site was planted on May 19, 1994 and consisted of 0.54 acres of transgenic corn (approximately 10,720 plants) harvested for yield determination, and 0.3 acres of transgenic corn (approximately 5,760 plants) that was evaluated for insect resistance. The trial was isolated by a distance of at least 660 feet from any other corn. The grain from all rows was harvested by machine on October 13, 1994. The grain was spread on the ground at the trial site, then tilled into the soil along with the remaining vegetative material. In the spring of 1995, the site will be replanted to a crop other than corn.

The second site was also planted on May 19, 1994 and consisted of 0.12 acres of transgenic corn (approximately 2,400 plants). Throughout the growing season, tissue samples were harvested for experimental purposes. The tassels of all plants were covered at the begining of pollen shed, then they were hand pollinated. The ears were hand harvested and some of the harvested seed was retained for further experimentation. Any seed not retained was spread over the plot site, then tilled into the soil along with the remaining vegetative material.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At four different stages the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodifiedcontrols.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not discloseany deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was chopped and returnedto the plot for soil composting.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-024-12N
Pioneer Number:	CORN-MO-94-11
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Telephone Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE
Gene efficacy.

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site at our research/breeding station located in Miami, Saline County, Missouri. The trial was planted on May 12, 1994, and comprised 0.50 acre. The trial was isolated by a distance of at least 660 feet from any other corn. Approximately 900 transgenic plants were evaluated for insect resistance. The grain from all rows was harvested by machine on October 1, 1994. All grain was destroyed and none was retained. In the spring of 1995, the site will be replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At one stage the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodifiedcontrols.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exihibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was chopped and returned to the plot for soil composting.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Rock County, WI

Permit Numbers 94-015XRA, 94-025-05n

Experiment Description Roundup Ready Corn - Field Efficacy Trial

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/9/94 **Harvest/Destroy Date** 7/28/94

Trial Size 0.43 acre

Corn Line Numbers 599-04-2

Field Trial Disposal Method Mowed

Isolation Method Used Destruction of plot prior to anthesis

Dates of Monitoring for Volunteer Corn Plants and Number Found
6/17/94, 7/27/94, 8/30/94 - None

[Golden Harvest Seed Company - Platteville] (LaFayette County), WI
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-033-04N phenotype: Lepidopteran insect resistance
94-033-05N phenotype: Lepidopteran insect resistant/Glyphosate tolerant
94-033-06N phenotype: Glyphosate tolerant

Monsanto: 94-031XRA,
94-032XRA
94-033XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress the gene of interest (GOI) into proprietary inbred lines. All three phenotypes identified above were planted and tested within the same field planting block.

The trial was planted May 4, 1994 over an area of 0.75 acres and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was detasseling of transgenic plants.

RESULTS

Adequate seed was produced to initiate the next cycle of backcrossing.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at harvest in 1994 and is continuing into the spring of 1995.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-03-2, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 604-09-1
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	762-03-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

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1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Hamilton County, Iowa

Permit Numbers 94-037XRA/94-038-01N
94-081XRA/94-082-08N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/19/94 **Harvest Date** 11/07/94

Trial Size 0.51 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned all grain and cobs harvested but not used in samples. Incorporated into the soil all unharvested grain, dropped ears and stalk material.

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/08/95 0

Method used to Destroy Volunteers: 1994 Plot area was seeded to alfalfa in 1995. 1994 plot area has been treated with benefin and imazethapyr and also has been mowed during 1995

Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Prowers County, CO

[CBI DELETED]

Permit Numbers 94-037XRA, 94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/18/94 **Harvest Date** 10/22/94

Trial Size 0.29 acres of tranagenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method Incorporated into the soil

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address	**Site Location**
[CBI DELETED]	Butler County, Iowa

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/18/94 **Harvest Date** 10/25/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

4/14/95	0	6/19/95	0
5/6/95	0	7/26/95	100

Method used to Destroy Volunteers: Herbicide treatment and mechanical cultivation

Other comments: Checked the field 7/31/95, there were no corn plants present

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address | **Site Location**

[CBI DELETED] | Des Moines County, Iowa

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/05/94 **Harvest Date** 10/04/94

Trial Size 0.28 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Incorporated into soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/20/95	0
06/01/95	6-8
06/06/95	8-10

Method used to Destroy Volunteers Disc

Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Henry County, Illinois

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/23/94 **Harvest Date** 10/1794

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Incorporated into soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

11/20/94	0	5/6/95	0
4/10/95	0	6/5/95	2-10 per 100 sq ft

Method used to Destroy Volunteers Disc plots twice, herb = treflan 1.5 pts + Scepter 2/3 pts, planted soybeans

Other comments 7/8/95 - 5/10 vol./100 sq ft. applied post (2 pts/A) - 7/18/95 cultivated 8/1/95 Destroyed remaining volunteers by hand weeding

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Clinton County, IL

[CBI DELETED]

Permit Numbers 94-037XRA, 94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/16/94 **Harvest Date** 10/12/94

Trial Size 0.300 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method All grain from plots was ground and deposited in a manure lagoon where it will rot.

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Warren County, IL

[CBI DELETED]

Permit Numbers 94-037XRA, 94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/10/94 **Harvest Date** 10/27/94

Trial Size .26 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method Mowed down. Incorporated into soil.

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address	**Site Location**
[CBI DELETED]	Hamilton County, Indiana

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/31/94 **Harvest Date** 10/27/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Incorporated into soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

04/30/95 0

Method used to Destroy Volunteers

Other comments Trial site will not be in crop during 1995 and is being used for a future building site.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address	**Site Location**
[CBI DELETED]	Chautauqua County, Kansas

Permit Numbers 94-037XRA/94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/15/94 **Harvest Date** 09/26/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Incorporated into soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

12/23/94	0	04/20/95	0	07/15/95	0
02/23/94	0	05/11/95	0		

Method used to Destroy Volunteers

Other comments No volunteer corn plants were detected at anytime. Entire plot area was disked on July 16, 1995, which would have destroyed any volunteer plants which might have existed but were undetected.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Ballard County, KY

[CBI DELETED]

Permit Numbers 94-037XRA, 94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/26/94 **Harvest Date** 10/7/94

Trial Size 0.220 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method Corn crop residue was destroyed by burning plus soil incorporation.

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Ottawa County, MI

[CBI DELETED]

Permit Numbers 94-037XRA, 94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/13/94 **Harvest Date** 10/27/94

Trial Size 0.275 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method Destroyed by mowing with subsequent incorporation into the soil.

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address	Site Location
[CBI DELETED]	Polk County, Minnesota

Permit Numbers 94-037XRA/94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/19/94 **Harvest Date** 11/14/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Chopped with forage harvester, returned corn residues to ground followed by a Bush Hog chopping then incorporated into the soil.

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

07/14/95 0

Method used to Destroy Volunteers Not applicable

Other comments 1995 crop is hard red spring wheat

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Rock County, Minnesota

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/23/94 **Harvest Date** 11/04/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/11/95	0	07/10/95	5
06/05/95	0	07/26/95	5

Method used to Destroy Volunteers Plants pulled and roots severed from shoots. Removed from plots.

Other comments Tilled 5/2/95 and approx. 6/1/95

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Shelby County, MO

[CBI DELETED]

Permit Numbers 94-037XRA, 94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/23/94 **Harvest Date** 10/13/94

Trial Size 0.290 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method Corn residue burned and incorporated into the soil.

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location York County, Nebraska

Permit Numbers 94-037XRA/94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/05/94 **Harvest Date** 09/28/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Incorporated into soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/06/95 0

Method used to Destroy Volunteers Soil was disked on 4/23/95

Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address	Site Location
[CBI DELETED]	Polk County, Nebraska

Permit Numbers 94-037XRA/94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/03/94 **Harvest Date** 09/29/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Incorporated into soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/01/95 0

Method used to Destroy Volunteers

Other comments Plots were disked on 04/28/95

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Fayette County Ohio

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/12/94 **Harvest Date** 10/20/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned grain and incorporated fodder into the soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

11/15/94	0
04/15/95	0
05/01/95	0

Method used to Destroy Volunteers

Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location LeHigh County
Pennsylvania

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/21/94 **Harvest Date** 10/12/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Mowed and disked into soil

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/11/95 0

Method used to Destroy Volunteers
Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address	Site Location
[CBI DELETED]	Minnehaha County South Dakota

Permit Numbers 94-037XRA, 94-038-OIN

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/23/94 **Harvest Date** 11/04/95

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Cut and will be incorporated into the soil in spring of 1995

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/15/95	0	07/05/95	0
05/05/95	0	07/26/95	0

Method used to Destroy Volunteers Not applicable

Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Hockley County Texas

Permit Numbers 94-037XRA/94-038-01N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/16/94 and 05/17/94 **Harvest Date** 09/27/94

Trial Size 0.29 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned excess grain and shredded and incorporated stubble into the soil.

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

10/27/94	7100	05/25/95	7100
04/28/95	7100	06/28/95	47

Method used to Destroy Volunteers

10/27/94	Area was disked
04/28/95	Area was rod weeded and hoed
05/25/95	Onion blade/rod weeder
06/28/95	All volunteers were displaying severe injury symptoms from Fusilado 2000 which had been sprayed on 06/20/95.

Other comments

FINAL REPORT
Kent Croon

Monsanto Company

Site Location	Champaign County, IL
Permit Numbers	94-047XRA, 94-055-18n
Comments	Nothing Planted at this Site Location

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Warren County, IL

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/12/94 **Harvest/Destroy Date** 10/14/94

Trial Size 0.63 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol

Isolation Method Used 660 feet isolation

Dates of Monitoring for Volunteer Corn Plants and Number Found
12/19/94, 3/8/95, 4/19/95, 5/16/95, 6/13/95, 7/14/95 - None

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Saunders County, NE

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description
Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn.

Results Trial was completed and observations taken as planned

General Field Observations
Several predators throughout field, a lot of Velvet weed and Pigweed were observed, however there were no difference noted between transgenic and non-transgenic plants. No disease was observed to be more or less severe in transgenic plants than in typical non-transgenic plants.

Planting Date 5/17/94 **Harvest/Destroy Date** 9/26/94

Trial Size 0.23 acre

Corn Line Numbers 599-04-2

Field Trial Disposal Method
Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol.

Isolation Method Used
660 feet isolation.

Monitoring for Volunteer Corn Plants and Number Found
6/28/95 and 7/20/95

Method Used to Destroy Volunteers
Chopped plants out manually.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Holt County, MO

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/26/94 **Harvest/Destroy Date** 12/8/94

Trial Size 0.63 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol

Isolation Method Used 660 feet isolation

Monitoring for Volunteers Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Saline County, MO

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Corn Smut - evaluated entire plot, however there were no differences noted between transgenic and non-transgenic plants

Planting Date 5/19/94 **Harvest/Destroy Date** 11/7/94

Trial Size 0.63 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol

Isolation Method Used 660 feet isolation

Monitoring for Volunteers Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Story County, IA

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn.

Results Trial was completed and observations taken as planned.

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Fusarium and Gibberella Stalk Rots were observed, however there were no differences noted between transgenic and non-transgenic plants related to disease occurrence. However, decreasing ECB damage appeared to result in decreased disease intensity.

Planting Date 5/14/94 **Harvest/Destroy Date** 10/15/94

Trial Size 1 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol.

Isolation Method Used 660 feet isolation

Dates of Monitoring for Volunteer Corn Plants and Number Found
5/27/95, 6/15/95, 6/30/95, 7/17/95 ~ 100

Method Used to Destroy Volunteers Cut with hoe

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Stafford County, KS

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/17/94 **Harvest/Destroy Date** 9/13/94

Trial Size 0.75 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol

Isolation Method Used 660 feet isolation

Dates of Monitoring for Volunteer Corn Plants and Number Found
6/28/95 - 0, 5/28/95 - 30,000+, 4/28/95 - 30, 10/13/94 - 0, 11/19/94 - 0

Method Used to Destroy Volunteers Field plowed

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Pemiscot County, MO

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/6/94 **Harvest/Destroy Date** 9/94

Trial Size 0.34 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol

Isolation Method Used 660 feet isolation

Dates of Monitoring for Volunteer Corn Plants and Number Found
4/1/95, 5/1/95, 6/1/95, 7/1/95 - 2

Method Used to Destroy Volunteers
Pulled by hand

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Tompkins County, NY

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results Trial was completed and observations taken as planned

General Field Observations Occasional Leaf Rust, Stalk and Smut was observed. Corn Rootworm were also observed, however there were no differences noted between transgenic and non-transgenic plants

Planting Date 5/20/94 **Harvest/Destroy Date** 11/7/94

Trial Size 0.5 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol

Isolation Method Used Removal of tassels

Dates of Monitoring for Volunteer Corn Plants and Number Found
3/29/95, 4/26/96, 5/16/95, 6/5/95, 7/3/95 - None

Method Used to Destroy Volunteers
Plowed Field 4/24/95

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Clay County, NE

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description
Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results
Trial was completed and observations taken as planned

General Field Observations
Chronic Sedge, Grassy weeds in 1st rep. were observed, however there were no differences noted between transgenic and non-transgenic plants, also storms have left plants ragged. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/18/94 **Harvest/Destroy Date** 10/10/94

Trial Size 0.23 acre

Corn Line Numbers 599-04-2, 572-24-1

Field Trial Disposal Method
Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol.

Isolation Method Used
660 feet isolation.

Dates of Monitoring for Volunteer Corn Plants
6/22/95 and 8/18/95

Method Used to Destroy Volunteers
Chopped plants out manually.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Dixon County, NE

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description
Study designed to investigate the impact of genetically modified corn on ECB and other insect pests endemic to corn. Study objective was to provide baseline information for the development of pest/resistance management strategies for integrating the B.t.k. Gene into existing Integrated Pest Management (IPM) practices in corn

Results
Trial was completed and observations taken as planned

General Field Observations
Several beneficials throughout field were observed, however there were no differences noted between transgenic and non-transgenic plants. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn.

Planting Date 5/19/94 **Harvest/Destroy Date** 10/17/94

Trial Size 0.23 acre

Corn Line Numbers 599-04-2

Field Trial Disposal Method
Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol.

Isolation Method Used
660 feet isolation.

Monitoring for Volunteer Corn Plants and Number Found
6/23/95 and 8/9/95

Method Used to Destroy Volunteers
Chopped plants out manually.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Saunders County, NE

Permit Numbers 94-047XRA, 94-055-18n

Experiment Description Weed Control Trial

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/26/94 **Harvest/Destroy Date** 8/11/94

Trial Size 0.33 acre

Corn Line Numbers 599-04-2

Field Trial Disposal Method Field trial was disposed by tillage into plot area as identified in relevant Experimental Use Permit (EUP) protocol

Isolation Method Used 660 feet isolation

Dates of Monitoring for Volunteer Corn Plants and Number Found
7/5/95 - 0

FINAL REPORT
Kent Croon

Monsanto Company

Site Location	Wake County, NC
Permit Numbers	94-047XRA, 94-055-18n
Comments	Nothing Planted at this Site Location

MONSANTO AG. COMPANY
SEED PRODUCTION

FINAL REPORT

Kent A. Croon
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-060-03N Lepidopteran insect resistance
Monsanto: 94-050XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to produce plant tissue including seed to support regulatory approval of corn lines containing the *B.t.k* protein.

Trials (0.32 A each, excluding alleyways) was planted at the Jerseyville, Illinois location on 5/19 and at the Monmouth, Illinois location on 5/20. The table on the next page lists the lines planted, arranged by vector. Plots were harvested at maturity on 9/26 (Jerseyville) and 10/4 (Monmouth).

The isolation method utilized was bagging of tassels shedding pollen in combination with shoot bags.

RESULTS

Seed and tissue production was as expected under Illinois conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1,576-01-1, 581-07-1, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 604-09-1

GENERAL FIELD OBSERVATIONS

No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. Transgenic plants did not demonstrate any evidence of the potential for increased weediness.

DISPOSAL

Remaining plant material after harvest was disked into the soil after harvest.

VOLUNTEERS

Monitoring for volunteers was initiated after harvest in 1994 and is continuing into the spring 1995 season.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Fairfax County, VA

Permit Numbers 94-067XRA, 94-074-09n

Experiment Description Efficacy demonstration trial

Results Trial was completed and observations taken as planned

General Field Observations All Btk - germinated. Only 3 of 72 Btk+ seeds did not germinate. No differences were noted between transgenic and non-transgenic plants

Planting Date 6/13/94 **Harvest/Destroy Date** 10/94

Trial Size 0.0013 acre

Corn Line Numbers 599-04-2

Field Trial Disposal Method Plant tissue returned to plot area

Isolation Method Used Removal of tassels

Dates of Monitoring for Volunteer Corn Plants and Number Found
11/7/94, 12/5/94, 1//9/95, 2/6/95, 3/6/95, 4/3/95, 5/8/95, 6/10/95, 7/10/95 - None

[CIBA Seed Company]
[Farmer City (McLean County), Illinois]
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-074-12N phenotype: Lepidopteran insect resistance
94-087-06N phenotype: Glyphosate tolerant

Monsanto: 94-070XRA
94-098XRA

EXPERIMENT DESCRIPTION

The objective of the trial was to determine the difference in yield between several pairs of lines, the pairs differing with respect to the presence or absence of the transgene of interest.

The trials were planted June 3, 1994, over an area of 0.23 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were mechanically harvested October 26, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial.

RESULTS

There was no significant difference in yield between most pairs of lines. Those lines where the gene(-) line yielded significantly more than the gene(+) were discontinued.

Vector	Lines
PV-ZMBK12L	749-01-1
PV-ZMBK16L	754-07-4, 748-04-3
PV-ZMCT01	481-10-1, 540-04-1, 544-03-2, 559-39-2, 572-16-1,
(PV-ZMBK07+PV-ZMGT10)	572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1,
	591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3cal, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic lines. Nothing unusual was noted as gene(+) transgenic plants were compared to gene(-) transgenic plants, indicating the gene per se was having no apparent pleiotropic effects. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Other than *Bt*(+) lines, no disease or insect damage was observed to be more or less severe in gene(+) transgenic plants than in gene(-) transgenic plants.

DISPOSAL

Remaining plant material after harvest was ground up and plowed into the soil after harvest.

VOLUNTEERS

Monitoring for volunteers was initiated in 1994 after the field season and will conclude this spring (1995).

[Golden Harvest Seed Company - Waterloo] (Douglas County), NE
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-074-14N phenotype: Lepidopteran insect resistance
94-087-08N phenotype: Glyphosate tolerant

Monsanto: 94-072XRA
94-100XRA

EXPERIMENT DESCRIPTION

The objective of the plantings was to assess the efficacy of the genes of interest (GOI) and to determine whether the inserted gene negatively affected agronomic characters such as yield and moisture. Both phenotypes identified above were tested in the same planting block and within the same isolation distance.

The trials were planted May 24, 1994 over an area of 1.4 acres and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was a 200 meter distance from other corn not part of this trial.

RESULTS

Most lines demonstrated excellent efficacy and no significant negative effect on yield or moisture. Those lines with less efficacy than desired, or having negative agronomic traits associated with the insertion were discontinued from further development.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at harvest in 1994 and is continuing into the spring of 1995.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 540-04-1, 544-03-2, 559-39-2, 572-16-1,
(PV-ZMBK07+PV-ZMGT10)	572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1ner,
	591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

[Golden Harvest Seed Company - Clinton] (DeWitt County), IL
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-074-14N phenotype: Lepidopteran insect resistance
94-087-08N phenotype: Glyphosate tolerant

Monsanto: 94-072XRA
94-100XRA

EXPERIMENT DESCRIPTION

The objective of the plantings was to assess the efficacy of the genes of interest (GOI), to determine whether the inserted gene negatively affected agronomic characters such as yield and moisture, and to introgress the GOI into proprietary inbred lines. Both phenotypes above were tested within the same field planting block.

The trials were planted May 16-18, 1994 over an area of 0.97 acres and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was detasseling of transgenic plants.

RESULTS

Most lines demonstrated excellent efficacy and no significant negative effect on yield or moisture. Those lines with less efficacy than desired, or having negative agronomic traits associated with the insertion were discontinued from further development. Adequate seed was produced to initiate the next cycle of backcrossing.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Except for the protection against ECB, no disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at harvest in 1994 and is continuing into the 1995 spring season.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-03-2, 554-03-2, 557-04-4, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1rus, 654-04-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 600-14-2, 604-09-1
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	762-03-1
PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	423-06-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

[Golden Harvest Seed Company - Henrietta] (Ray County), MO
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-074-14N phenotype: Lepidopteran insect resistant
94-087-08N phenotype: Glyphosate tolerant

Monsanto: 94-O72XRA
94-100XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress genes of interest (GOI) into proprietary inbred lines. Both phenotypes identified above were tested in the same planting block.

The trial was planted May 17, 1994 over an area of 0.11 acres and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was detasseling of transgenic plants.

RESULTS

Adequate seed was produced to initiate the next cycle of backcrossing.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at the end of harvest in 1994 and is continuing into 1995.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-03-2, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 576-01-1, 581-07-1ner, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 604-09-1
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	762-03-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

JERSEYVILLE, ILLINOIS FIELD TRIALS
1994 GROWING SEASON

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA:	94-082-03N	phenotype: Lepidopteran insect resistance
	94-084-13N	phenotype: Glyphosate tolerant
	94-116-03N	phenotype: Lepidopteran insect resistance
	94-116-04N	phenotype: Glyphosate tolerant
	94-118-03N	phenotype: Glyphosate tolerant/Carbohydrate metabolism
Monsanto:	94-073XRA	
	94-091XRA	
	94-127XRA	
	94-128XRA	
	94-132XRA	

EXPERIMENT DESCRIPTIONS

Several protocols were established under these notifications. Objectives included determination of the efficacy of the gene of interest (GOI), seed increases and gene introgression into elite inbred lines, assessments of effects of the inserted gene on agronomic traits, demonstrations of gene efficacy, and assessment of weed control options with the availability of Roundup-Ready™ corn. Both lead lines and new transformation events for each of the above phenotypes were tested in the same testing block to facilitate comparison. All lines were contained within the same isolation.

The trials were established at the Monsanto Research Farm in Jerseyville, Illinois, in Jersey County. The transgenic lines planted in these trials are listed in Table 1. Trials were planted over the period 5/18/94 - 6/4/94, and harvested at maturity. Plots were isolated at least 200 meters from any other corn not part of these trials that was allowed to go to flower. Total transgenic area of the trials was 3.7 acres.

RESULTS

For previously tested lines, efficacy was confirmed in most cases; those lines not demonstrating adequate protection were discontinued. Similarly, lines evidencing agronomic deficiencies such as lower yield or higher moisture attributable to the GOI were discontinued from further developments. New lines were compared to previously selected lines for efficacy. Several lines were identified that had similar efficacy, and will be evaluated again. Seed production was excellent, and comparable to non-transgenic versions of the particular recurrent parent. Demonstration plots reflected the excellent protection afforded by the particular GOI included. Weed control studies demonstrated the excellent potential of Roundup-Ready™ corn plus Roundup® to be an important component of weed control practices in corn in the future.

GENERAL FIELD OBSERVATIONS

Transgenic lines were compared to either related non-transgenic inbreds or hybrids or to sibs that did not have the GOI. No differences were noted in disease or insect susceptibility. No line exhibited any characteristic that might lead to increased weediness, such as excessive tillering, seed shattering, or a perennial habit. One line was observed to have the tassel seed characteristic apparently associated with the presence of the gene; this line was dropped from further field evaluations.

FIELD TRIAL DISPOSAL METHOD

Following harvest, plots were chopped and residue incorporated into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated in the fall of 1994 and volunteers destroyed. Monitoring is continuing into the spring 1995 season.

Table 1. List of transgenic lines tested in Jerseyville, IL, during the 1994 growing season, arranged by vector used in the transformation

PV-ZMBK10
631-03-1

PV-ZMBK10L
1001-10-1, 958-12-2, 989-02-2, 989-20-2, 990-07-1, 990-07-2, 995-03-1, 997-16-5, 997-19-12, 997-19-4, 997-20-1, 997-21-5, 997-27-1, 997-27-2, 997-27-7

PV-ZMBK12
719-03-1, 792-10-1

PV-ZMBK12L
1000-10-1, 1005-06-1, 1006-03-1, 1006-07-2, 1006-08-1, 1014-04-1, 1014-16-1, 1016-06-2, 1018-01-2, 1018-09-2, 749-01-1, 976-02-3, 976-08-1, 976-08-3, 977-02-2, 978-02-2, 992-09-1, 992-14-1

PV-ZMBK16
742-10-1, 764-06-2, 774-16-1

PV-ZMBK16L
746-02-2, 748-04-3, 754-07-4, 754-10-1, 774-02-2, 789-14-1

PV-ZMBK20L
1014-03-2, 1016-03-1, 1016-07-2, 1016-11-1, 1018-06-1, 1032-03-2, 1032-06-11, 1032-06-14, 1032-07-3, 1032-07-4, 1032-07-5, 1032-07-7, 1032-08-1, 1032-08-2, 1032-08-6, 1032-11-9, 1032-12-5, 1032-13-1, 1032-13-14, 1032-15-2, 1032-16-13, 1032-16-14, 1032-16-18, 1032-17-2, 1032-18-1, 1032-18-11, 1032-18-16, 1032-18-17, 1032-19-11, 1032-19-13, 1032-19-14, 1032-19-16, 1032-19-3, 1032-21-1, 1032-21-6, 1032-22-3, 1032-22-4, 1032-22-5, 1033-01-2, 1033-01-4, 1033-06-1, 1033-06-5, 1033-09-2, 1033-16-2, 1033-19-1, 1033-22-2, 1033-27-1, 1034-07-1, 1034-17-1

PV-ZMBK21L
1171-11-4, 1171-17-4, 1171-18-1

PV-ZMGT05L
1102-03-1, 1104-04-1, 1104-06-2, 1104-09-2, 1105-07-2, 1137-04-1, 1137-10-1, 1150-02-2, 1151-12-2

PV-ZMGT10
1073-07-2

PV-ZMGT10L
1023-04-1, 1025-05-1, 1025-06-1, 1025-06-3, 1027-08-1, 1028-02-1, 1031-05-2, 1056-08-1, 1059-01-1, 1061-05-2, 1072-10-2, 1082-06-1, 1096-02-1, 1115-01-1, 1115-01-3, 1115-06-3, 1115-07-3, 1115-07-6, 1115-07-7, 870-02-2, 936-09-2

PV-ZMGT15L
1055-08-2, 1062-07-2, 1062-09-2, 1062-09-3, 1062-11-4, 1062-11-5, 1071-01-3, 1071-05-4, 1071-07-1, 1078-01-3, 1078-03-1, 1078-03-2, 1078-03-3, 1083-01-1, 1111-05-1, 1111-09-2, 1112-03-1, 1114-02-7, 1114-03-4, 1114-04-3, 1114-04-5, 1114-04-6, 1114-04-7, 1114-07-3, 1114-07-5, 1167-08-1, 1170-04-1, 1170-08-1

PV-ZMGT16L

041-02-1, 1041-04-7, 1041-10-1, 1041-10-3, 1041-10-6, 1045-02-2, 1046-03-1, 1046-10-1, 1049-03-2, 1049-11-1, 1052-02-1, 1053-03-1, 1053-07-1, 1056-01-1, 1056-06-1, 1056-06-2, 1057-07-2, 1057-10-1, 1059-08-3, 1061-02-2, 1061-03-1, 1061-06-9, 1061-07-2, 1063-02-1, 1063-03-3, 1071-08-2, 1081-04-2, 1082-05-1, 1082-05-3, 1083-04-1, 1083-08-6, 1083-08-7, 1090-01-3, 1090-04-4, 1090-07-1, 1090-07-3, 1091-04-6, 1091-05-1, 1091-09-1, 1091-09-2, 1091-09-5, 1093-04-1, 1093-04-2, 1093-05-1, 1093-05-3, 1093-05-5, 1093-09-1, 1093-09-4, 1093-12-1, 1093-12-2, 1093-12-3, 1093-12-5, 1095-03-4, 1095-03-5, 1095-03-6, 1095-05-2, 1095-07-3, 1095-13-2, 1096-09-1, 1096-10-3, 1096-13-2

PV-ZMGT17L

1090-05-2, 1091-01-1, 1091-02-1, 1091-06-2, 1093-02-1, 1093-02-4, 1093-03-1, 1093-08-8, 1093-11-3, 1102-02-1, 1102-08-2, 1103-01-3, 1103-12-1, 1104-05-3, 1162-03-1, 1162-03-2, 1162-10-6, 1162-13-4, 1163-06-1, 1163-06-3

PV-ZMGT18L

1111-03-6, 1112-02-1, 1112-05-1, 1114-05-4, 1114-06-1, 1137-03-1, 1151-09-1, 1157-11-1, 1162-02-1, 1162-11-4, 1162-12-2, 1163-07-1, 1163-07-2, 1163-12-1, 1170-07-1

PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)

481-10-1, 540-04-1, 544-03-2, 554-03-2, 557-04-4, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1, 654-04-1, 658-06-1

PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)

599-04-2, 599-04-3, 600-14-2, 604-09-1

PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)

462-03-2

PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)

762-03-1

PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)

423-06-1

PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)

766-07-1, 835-10-1, 837-02-1, 842-02-1, 842-03-1, 842-05-1, 842-06-2, 856-03-2

PV-ZMCT15 (PV-ZMGT15+PV-ZMSM07)

811-05-1, 811-22-1, 818-05-2, 818-05-3, 818-06-2, 820-07-5, 822-09-1, 826-01-4, 829-02-2, 829-10-4, 829-12-3, 834-09-1, 836-06-1

PV-ZMCT16 (PV-ZMGT11+PV-ZMSM08)
849-02-1, 850-01-2, 851-01-2, 854-03-1, 855-03-1, 858-04-1

PV-ZMCT17 (PV-ZMGT01+V-ZMGT03)
788-03-3

PV-ZMMT01 (PV-ZMBK25+PV-ZMSM06+PV-ZMSM10)
236-07-4, 236-08-10

PV-ZMCT31 (PV-ZMHS06+PV-ZMGT10)
884-07-1, 972-22-1

PV-ZMCT36 (PV-ZMHS03+PV-ZMGT10)
893-09-1

PV-ZMCT38 (PV-ZMHS04+V-ZMGT10)
889-01-1

MONMOUTH, ILLINOIS FIELD TRIALS
1994 GROWING SEASON

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA:	94-082-03N	phenotype: Lepidopteran insect resistance
	94-084-13N	phenotype: Glyphosate tolerant
	94-116-03N	phenotype: Lepidopteran insect resistance
	94-116-04N	phenotype: Glyphosate tolerant
	94-118-03N	phenotype: Glyphosate tolerant/Carbohydrate metabolism

Monsanto: 94-073XRA
94-091XRA
94-127XRA
94-128XRA
94-132XRA

EXPERIMENT DESCRIPTIONS

Several trials were conducted under these notifications. Objectives included determination of the efficacy of the gene of interest (GOI), seed increases and gene introgression into elite inbred lines, assessments of effects of the inserted gene on agronomic traits, and assessment of weed control options with the availability of Roundup-Ready™ corn. Both lead lines and new transformation events for each of the above phenotypes were tested in the same testing block to facilitate comparison. All lines were contained within the same isolation.

The trials were established at the Monsanto Research Farm in Monmouth, Illinois, in Warren County. The transgenic lines planted in these trials are listed in Table 1. Trials were planted over the period 5/25/94 - 6/10/94, and harvested at maturity. Plots were isolated at least 200 meters from any other corn not part of these trials that was allowed to go to flower. Total transgenic area of the trials was 3.0 acres.

RESULTS

For previously tested lines, efficacy was confirmed in most cases; those lines not demonstrating adequate protection were discontinued. Similarly, lines evidencing agronomic deficiencies such as lower yield or higher moisture attributable to the GOI were discontinued from further developments. New lines were compared to previously selected lines for efficacy. Several lines were identified that had similar efficacy, and will be evaluated again. Seed production was excellent, and comparable to non-transgenic versions of the particular recurrent parent. Weed control studies demonstrated the excellent potential of Roundup-Ready™ corn plus Roundup® to be an important component of weed control practices in corn in the future.

GENERAL FIELD OBSERVATIONS

Transgenic lines were compared to either related non-transgenic inbreds or hybrids or to sibs that did not have the GOI. No differences were noted in disease or insect susceptibility. No line exhibited any characteristic that might lead to increased weediness, such as excessive tillering, seed shattering, or a perennial habit. One line was observed to have the tassel seed characteristic apparently associated with the presence of the gene; this line was dropped from further field evaluations.

FIELD TRIAL DISPOSAL METHOD

Following harvest, plots were chopped and residue incorporated into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated in the fall of 1994 and volunteers destroyed. Monitoring is continuing into the spring 1995 season.

Table 1. List of transgenic lines tested in Monmouth, IL, during the 1994 growing season, arranged by vector used in the transformation

PV-ZMBK10
631-03-1

PV-ZMBK10L
1001-10-1, 958-12-2, 990-07-1, 990-07-2, 995-03-1, 997-16-5, 997-19-12, 997-19-4, 997-21-5, 997-27-1, 997-27-7

PV-ZMBK12
792-10-1

PV-ZMBK12L
1000-10-1, 1005-06-1, 1006-03-1, 1006-07-2, 1006-08-1, 1014-04-1, 1018-09-2, 749-01-1, 976-02-3, 976-08-1, 976-08-3, 977-02-2, 992-09-1, 992-14-1

PV-ZMBK16
774-16-1

PV-ZMBK16L
746-02-2, 748-04-3, 754-07-4, 754-10-1, 774-02-2

PV-ZMBK20L
1016-03-1, 1032-03-2, 1032-07-3, 1032-07-4, 1032-08-1, 1032-08-2,
1032-08-6, 1032-12-5, 1032-13-1, 1032-13-14, 1032-15-2, 1032-16-13,
1032-16-14, 1032-16-18, 1032-17-2, 1032-18-11, 1032-18-16,
1032-18-17, 1032-19-11, 1032-19-13, 1032-19-14, 1032-19-16,
1032-19-3, 1032-21-1, 1032-21-6, 1033-06-1, 1033-06-5, 1033-09-2,
1033-16-2, 1033-19-1, 1033-22-2, 1034-07-1, 1034-17-1

PV-ZMGT05L
1104-06-2, 1105-07-2

PV-ZMGT10L
1023-04-1, 1025-05-1, 1025-06-1, 1025-06-3, 1028-02-1, 1031-05-2,
1056-08-1, 1061-05-2, 1115-01-1, 1115-01-3

PV-ZMGT15L
1062-07-2, 1078-03-1, 1114-02-7, 1114-03-4

PV-ZMGT16L
1041-04-7, 1041-10-1, 1041-10-3, 1041-10-6, 1045-02-2, 1046-03-1,
1046-10-1, 1049-03-2, 1053-03-1, 1056-06-1, 1057-07-2, 1057-10-1,
1059-08-3, 1061-06-9, 1061-07-2, 1063-03-3, 1090-01-3, 1090-07-1,
1091-09-1, 1093-12-2, 1095-03-5, 1095-13-2, 1096-10-3

PV-ZMGT17L
091-02-1, 1093-02-1, 1093-03-1, 1103-12-1, 1162-03-1

PV-ZMGT18L
1112-02-1, 1157-11-1, 1162-02-1, 1163-07-1, 1163-12-1, 1170-07-1

PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)
481-10-1, 540-04-1, 544-03-2, 554-03-2, 557-04-4, 559-39-2,
572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1,
591-03-2, 639-02-1, 654-04-1, 658-06-1

PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)
599-04-2, 599-04-3, 600-14-2, 604-09-1, 618-40-1

PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)
462-03-2

PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)
762-03-1

PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)
423-06-1

PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)
766-07-1, 835-10-1, 837-02-1, 842-02-1, 842-03-1, 842-06-2, 856-03-2

PV-ZMCT15 (PV-ZMGT15+PV-ZMSM07)
811-22-1, 818-05-2, 818-05-3, 822-09-1, 826-01-4, 829-02-2, 829-10-4, 829-12-3, 834-09-1, 836-06-1

PV-ZMCT16 (PV-ZMGT11+PV-ZMSM08)
849-02-1, 850-01-2, 851-01-2, 855-03-1, 858-04-1

PV-ZMCT31 (PV-ZMHS06+PV-ZMGT10)
884-07-1, 972-22-1

PV-ZMCT38 (PV-ZMHS04+PV-ZMGT10)
889-01-1

PV-ZMCT36 (PV-ZMHS03+PV-ZMGT10)
893-09-1

[Holden Foundation Seed Company - Williamsburg] (Iowa County), IA
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-082-04N phenotype: Lepidopteran insect resistance
94-087-09N phenotype: Glyphosate tolerant

Monsanto: 94-086XRA
94-101XRA

EXPERIMENT DESCRIPTION

The objective of the plantings was to assess the efficacy of the genes of interest (GOI), introgress the GOI into proprietary inbred lines, and to determine whether the inserted gene negatively affected agronomic characters such as yield and moisture. Both phenotypes identified above were planted into the same research testing block.

The trials were planted May 27 - June 1, 1994, covering 1.19 acres, and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was separation of at least 200 meters from non-transgenic corn not part of the trials.

RESULTS

Most lines demonstrated excellent efficacy and no significant negative effect on yield or moisture. Those lines with less efficacy than desired, or having negative agronomic traits associated with the insertion were discontinued from further development. Adequate seed was produced to initiate the next cycle of backcrossing.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Except for the protection against ECB, no disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at harvest in 1994 and is continuing in the spring of 1995.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 513-11-2, 540-04-1, 544-03-2, 554-03-2,
(PV-ZMBK07+PV-ZMGT10)	557-04-4, 559-39-2, 572-16-1, 572-24-1, 574-04-2,
	575-07-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1,
	654-04-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 600-14-2, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT07	423-06-1
(PV-ZMGT03+PV-ZMGT05)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

[Holden Foundation Seed Company - Franklin] (Johnson County), IN
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-082-04N phenotype: Lepidopteran insect resistant
94-087-09N phenotype: Glyphosate tolerant

Monsanto: 94-086XRA
94-101XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to determine whether the inserted gene negatively affected agronomic characters such as yield and moisture. Both phenotypes identified above were tested within the same research block at the site and within the same isolation distance.

The trial was planted May 27, 1994, covering 0.23 acres, and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was separation of at least 200 meters from non-transgenic corn not part of the trials.

RESULTS

Most lines showed no significant negative effect on yield or moisture. Those lines having negative agronomic traits associated with the insertion were discontinued from further development.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Except for the protection against ECB, no disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers is wasinitiated at harvest in 1994 and is continuing into the spring of 1995.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 540-04-1, 544-03-2, 559-39-2, 572-16-1,
(PV-ZMBK07+PV-ZMGT10)	572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1,
	591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

[Limagrain Seed Company]
[Champaign] (Champaign County), Illinois
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-082-05N phenotype: Lepidopteran insect resistance
94-087-11N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 94-085XRA
94-103XRA

EXPERIMENT DESCRIPTION

The objective of this planting was to introgress genes of interest into proprietary inbredlines. As part of this process, lines were screened for efficacy conferred by genes coding for *Bt* insecticidal protein and/or tolerance to glyphosate. Both corn phenotypic traits identified above were tested in the same field testing block.

The trial was planted May 18, 1994, over an area of 0.14 acres. The table below lists the lines planted, arranged by vector.

Selected ears were hand harvested September 16, 1994.

The isolation method utilized was detasselling transgenic plots.

RESULTS

Seed production was as expected. Using European corn borers or glyphosate as appropriate, positive plants were identified to continue the introgression process.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-03-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 604-09-1,
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	762-03-1

GENERAL FIELD OBSERVATIONS

Plots were observed five times during the growing season, from emergence to harvest. No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents.

DISPOSAL

Ears not harvested were collected and burned on site, and the ashes spread within plot boundaries. The ahes and remaining plant material was then chopped and chisel-plowed.

VOLUNTEERS

The monitoring is ongoing. Thus far, no volunteer corn has been observed.

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1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Clinton County, IL

[CBI DELETED]

Permit Numbers 94-081XR, 94-082-0[illegible]N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/16/94 **Harvest Date** 10/12/94

Trial Size 0.300 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method All grain from plots was ground and deposited in a manure lagoon where it will rot.

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Hamilton County, Iowa

Permit Numbers 94-037XRA/94-038-01N
94-081XR/94-082-08N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/19/94 **Harvest Date** 11/07/94

Trial Size 0.51 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned all grain and cobs harvested but not used in samples. Incorporated into the soil all unharvested grain, dropped ears and stalk material.

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/08/95 0

Method used to Destroy Volunteers: 1994 Plot area was seeded to alfalfa in 1995. 1994 plot area has been treated with benefin and imazethapyr and also has been mowed during 1995

Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Hamilton County, Iowa

Permit Numbers 94-037XRA/94-038-01N
94-081XRA/94-082-08N

Experiment Description 94-081XR

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/19/94 **Harvest Date** 11/07/94

Trial Size 0.51 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned all grain and cobs harvested but not used in samples. Incorporated into the soil all unharvested grain, dropped ears and stalk material.

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/08/95 0

Method used to Destroy Volunteers: 1994 Plot area was seeded to alfalfa in 1995. 1994 plot area has been treated with benefin and imazethapyr and also has been mowed during 1995

Other comments

[CARGILL Seed Company]
[Aurora (Kane County), Illinois]
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-082-10N phenotype: Lepidopteran insect resistance
94-087-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 94-083XRA
94-096XRA

EXPERIMENT DESCRIPTION

The objectives of these trials were to: (1) determine the difference in yield between several pairs of lines, the pairs differing with respect to the presence or absence of the transgene of interest; (2) evaluate for tolerance to different application regimes of Roundup® herbicide; (3) evaluate for tolerance to feeding damage from European Corn Borer (ECB); and (4) continue introgression of genes into proprietary inbred lines. Both corn phenotypic traits identified above were tested in the same field testing block.

The trials were planted May 18-19, 1994, over an area of 1.5 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were mechanically harvested October 25-26, 1994.

The isolation methods utilized were at least 200 meter isolation from open-pollinated corn not part of this trial.

RESULTS

Objective 1: There was no significant difference in yield between most pairs of lines. Those lines where the gene(-) line yielded significantly more than the gene(+) were discontinued. Objective 2: Lines were identified that appeared to have commercial levels of glyphosate tolerance. Objective 3: All *Bt* lines evaluated adequately controlled ECB. Objective 4: Adequate seed was produced to continue the next generation.

Vector	Lines
PV-ZMBK10	631-03-1, 634-11-1, 714-05-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 54-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 523-06-1, 540-04-1, 544-03-2, 546-09-1, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 604-09-1
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	62-03-1
PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	402-08-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic lines. Nothing unusual was noted as gene(+) transgenic plants were compared to gene(-) transgenic plants, indicating the gene per se was having no apparent pleiotropic effects. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Other than in Bt(+) plants, no disease or insect damage was observed to be more or less severe in gene(+) transgenic plants than in gene(-) transgenic plants.

DISPOSAL

Remaining plant material after harvest was ground up and plowed into the soil after harvest.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into the 1995 spring season.

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1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address **Site Location** Clinton County, IL

[CBI DELETED]

Permit Numbers 94-081XR, 94-082-0[illegible]N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 5/16/94 **Harvest Date** 10/12/94

Trial Size 0.300 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method All grain from plots was ground and deposited in a manure lagoon where it will rot.

Isolation method used 200 meter isolation

Monitoring for volunteer corn plants
Plot areas were monitored for volunteer corn plants and fields appropriately rotated into alternate crops.

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Hamilton County, Iowa

Permit Numbers 94-037XRA/94-038-01N
94-081XR/94-082-08N

Experiment Description

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/19/94 **Harvest Date** 11/07/94

Trial Size 0.51 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned all grain and cobs harvested but not used in samples. Incorporated into the soil all unharvested grain, dropped ears and stalk material.

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/08/95 0

Method used to Destroy Volunteers: 1994 Plot area was seeded to alfalfa in 1995. 1994 plot area has been treated with benefin and imazethapyr and also has been mowed during 1995

Other comments

1994 Corn Magnitude of the Residue Trials

Final Report

Mark Oppenhuizen
Monsanto Company

Cooperator Name and Address [CBI DELETED]

Site Location Hamilton County, Iowa

Permit Numbers 94-037XRA/94-038-01N
94-081XRA/94-082-08N

Experiment Description 94-081XR

The objective of the plantings was to determine the magnitude of the glyphosate residues in or on the plants following the proposed label guidelines. Plant samples were taken at harvest and at various other times from the plots, frozen, and shipped to Monsanto for analytical analysis. These studies were conducted in accordance with Good Laboratory Practices (40 CFR 160).

Results

The site yielded satisfactory samples for glyphosate analysis.

General Field Observations

Since the purpose of this trial was specific for the purposes of the magnitude of the residue trial, no additional information on insect or disease susceptibiity, weediness potential, or growth characteristics were requested by the protocol or recorded.

Planting Date 05/19/94 **Harvest Date** 11/07/94

Trial Size 0.51 acres of transgenic plants

Corn Line Numbers 599-04-2, 572-24-1

Field trial disposal method: Burned all grain and cobs harvested but not used in samples. Incorporated into the soil all unharvested grain, dropped ears and stalk material.

Isolation method used 200 meter isolation

Dates of monitoring for volunteer corn plants and number found

05/08/95 0

Method used to Destroy Volunteers: 1994 Plot area was seeded to alfalfa in 1995. 1994 plot area has been treated with benefin and imazethapyr and also has been mowed during 1995

Other comments

[CARGILL Seed Company]
[Sugar Grove (Kane County), Illinois]
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-082-10N phenotype: Lepidopteran insect resistance
94-087-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 94-083XRA
94-096XRA

EXPERIMENT DESCRIPTION

The objectives of these trials were to: (1) determine the difference in yield between several pairs of lines, the pairs differing with respect to the presence or absence of the transgene of interest; (2) evaluate for tolerance to different application regimes of Roundup® herbicide; and (3) evaluate for tolerance to feeding damage from European Corn Borer (ECB). Both corn phenotypic traits identified above were tested in the same field testing block.

The trials were planted May 18-19, 1994, over an area of 0.56 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were mechanically harvested October 25-26, 1994.

The isolation methods utilized was temporally shifting the planting so anthesis would not correspond to silking of adjacent corn.

RESULTS

Objective 1: There was no significant difference in yield between most pairs of lines. Those lines where the gene(-) line yielded significantly more than the gene(+) were discontinued. Objective 2: Lines were identified that appeared to have commercial levels of glyphosate tolerance. Objective 3: All *Bt* lines evaluated adequately controlled ECB.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 54-10-1
PV-ZMCT01	481-10-1, 523-06-1, 540-04-1, 544-03-2, 559-39-2,
(PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1,
	581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	62-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic lines. Nothing unusual was noted as gene(+) transgenic plants were compared to gene(-) transgenic plants, indicating the gene per se was having no apparent pleiotropic effects. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Other than in Bt(+) plants, no disease or insect damage was observed to be more or less severe in gene(+) transgenic plants than in gene(-) transgenic plants.

DISPOSAL

Remaining plant material after harvest was plowed into the soil after harvest. Harvested seed was brought to Aurora, IL site where it was spread out over the transgenic plot area there and disked in.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into 1995.

[CARGILL Seed Company]
[Monticello (Piatt County), Illinois]
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-082-10N phenotype: Lepidopteran insect resistance
94-087-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 94-083XRA
94-096XRA

EXPERIMENT DESCRIPTION

The objective of these trials was to determine the difference in yield between several pairs of lines, the pairs differing with respect to the presence or absence of the transgene of interest. Both corn phenotypic traits identified above were tested in the same field testing block.

The trial was planted May 19, 1994, over an area of 0.24 acres. The table below lists the lines planted, arranged by vector.

Plots were mechanically harvested November 3, 1994.

The isolation method utilized was detasselling transgenic plots. Non-transgenic pollinator rows were used to supply pollen for yield measurements.

RESULTS

There was no significant difference in yield between most pairs of lines. Those lines where the gene(-) line yielded significantly more than the gene(+) were discontinued.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 54-10-1
PV-ZMCT01	481-10-1, 523-06-1, 540-04-1, 544-03-2, 559-39-2,
(PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1,
	581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	62-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic lines. Nothing unusual was noted as gene(+) transgenic plants were compared to gene(-) transgenic plants, indicating the gene per se was having no apparent pleiotropic effects. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Other than in Bt(+) plants, no disease or insect damage was observed to be more or less severe in gene(+) transgenic plants than in gene(-) transgenic plants.

DISPOSAL

Remaining plant material after harvest was plowed into the soil after harvest. Harvested seed was brought to Aurora, IL site where it was spread out over the transgenic plot area there and disked in.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into the 1995 spring season.

[CARGILL Seed Company]
[Grinnell (Poweshiek County), Iowa]
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-082-10N phenotype: Lepidopteran insect resistance
94-087-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 94-083XRA
94-096XRA

EXPERIMENT DESCRIPTION

The objective of these trials was to determine the difference in yield between several pairs of lines, the pairs differing with respect to the presence or absence of the transgene of interest. Both corn phenotypic traits identified above were tested in the same field testing block.

The trial was planted May 24, 1994, over an area of 0.24 acres. The table below lists the lines planted, arranged by vector.

Plots were mechanically harvested October 24-29, 1994.

The isolation method utilized was detasselling transgenic plots. Non-transgenic pollinator rows were used to supply pollen for yield measurements.

RESULTS

There was no significant difference in yield between most pairs of lines. Those lines where the gene(-) line yielded significantly more than the gene(+) were discontinued.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, , 54-10-1
PV-ZMCT01	481-10-1, 523-06-1, 540-04-1, 544-03-2, 559-39-2,
(PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1,
	581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	62-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic lines. Nothing unusual was noted as gene(+) transgenic plants were compared to gene(-) transgenic plants, indicating the gene per se was having no apparent pleiotropic effects. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Other than in *Bt*(+) plants, no disease or insect damage was observed to be more or less severe in gene(+) transgenic plants than in gene(-) transgenic plants.

DISPOSAL

Remaining plant material after harvest was plowed into the soil after harvest.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into the 1995 spring season.

[CARGILL Seed Company]
[Covington (Miami County), Ohio]
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-082-10N phenotype: Lepidopteran insect resistance
94-087-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 94-083XRA
94-096XRA

EXPERIMENT DESCRIPTION

The objective of these trials was to determine the difference in yield between several pairs of lines, the pairs differing with respect to the presence or absence of the transgene of interest. Both corn phenotypic traits identified above were tested in the same field testing block.

The trial was planted May 17, 1994, over an area of 0.24 acres. The table below lists the lines planted, arranged by vector.

Plots were mechanically harvested October 31, 1994.

The isolation method utilized was detasselling transgenic plots. Non-transgenic pollinator rows were used to supply pollen for yield measurements.

RESULTS

There was no significant difference in yield between most pairs of lines. Those lines where the gene(-) line yielded significantly more than the gene(+) were discontinued.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 54-10-1
PV-ZMCT01	481-10-1, 523-06-1, 540-04-1, 544-03-2, 559-39-2,
(PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1,
	581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	62-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic lines. Nothing unusual was noted as gene(+) transgenic plants were compared to gene(-) transgenic plants, indicating the gene per se was having no apparent pleiotropic effects. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Other than in Bt(+) plants, no disease or insect damage was observed to be more or less severe in gene(+) transgenic plants than in gene(-) transgenic plants.

DISPOSAL

Remaining plant material after harvest was plowed into the soil after harvest.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into the 1995 spring season.

[CARGILL Seed Company]
[Carrollton (Carroll County), Missouri]
SUMMER, 1994 TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-082-10N phenotype: Lepidopteran insect resistance
94-087-04N phenotype: Lepidopteran insect resistant/Glyphosate tolerant

Monsanto: 94-083XRA
94-096XRA

EXPERIMENT DESCRIPTION

The objective of these trials was to determine the difference in yield between several pairs of lines, the pairs differing with respect to the presence or absence of the transgene of interest. Both corn phenotypic traits identified above were tested in the same field testing block.

The trial was planted May 19, 1994, over an area of 0.24 acres. The table below lists the lines planted, arranged by vector.

Plots were mechanically harvested October 18, 1994.

The isolation method utilized was detasselling transgenic plots. Non-transgenic pollinator rows were used to supply pollen for yield measurements.

RESULTS

There was no significant difference in yield between most pairs of lines. Those lines where the gene(-) line yielded significantly more than the gene(+) were discontinued.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 54-10-1
PV-ZMCT01	481-10-1, 523-06-1, 540-04-1, 544-03-2, 559-39-2,
(PV-ZMBK07+PV-ZMGT10)	572-16-1, 572-24-1, 574-04-2, 575-07-2, 576-01-1,
	581-07-1, 591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	62-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

There were no unexpected phenotypes. While there were differences in plant phenotype, these could be attributed solely to differences in genetic background of the transgenic lines. Nothing unusual was noted as gene(+) transgenic plants were compared to gene(-) transgenic plants, indicating the gene per se was having no apparent pleiotropic effects. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Other than in Bt(+) plants, no disease or insect damage was observed to be more or less severe in gene(+) transgenic plants than in gene(-) transgenic plants.

DISPOSAL

Remaining plant material after harvest was burned.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into the 1995 spring season.

[ASGROW Seed Company - Stonington] (Christian County), IL
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-083-02N phenotype: Lepidopteran insect resistance
94-084-17N phenotype: Glyphosate tolerant

Monsanto: 94-088XRA
94-095XRA

EXPERIMENT DESCRIPTION

The objective of the plantings was to assess the efficacy of the genes of interest (GOI) and to determine whether the inserted gene negatively affected agronomic characters such as yield and moisture. Both phenotypes identified above were tested at the same location in the same testing block.

The trials were planted June 17, 1994, covering 2.0 total acres, and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was temporal shift, assuring no receptive silks of corn within 200 meters.

RESULTS

Most lines demonstrated excellent efficacy and no significant negative effect on yield or moisture. Those lines with less efficacy than desired, or having negative agronomic traits associated with the insertion were discontinued from further development.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Except for the protection against European corn borer (ECB), no disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at harvest in 1994 and is continuing into the 1995 spring season.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 540-04-1, 544-03-2, 559-39-2, 572-16-1,
(PV-ZMBK07+PV-ZMGT10)	572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1,
	591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

[ICI Seeds - Slater] (Boone County), IA
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-083-03N phenotype: Lepidopteran insect resistance
94-087-10N phenotype: Glyphosate tolerant

Monsanto: 94-089XRA
94-102XRA

EXPERIMENT DESCRIPTION

The objective of the plantings was to assess the efficacy of the genes of interest (GOI), introgress the GOI into proprietary inbred lines, and to determine whether the inserted gene negatively affected agronomic characters such as yield and moisture. Both phenotypes were planted in the same research testing block.

The trials were planted May 17 - May 31, 1994, covering 0.487 acres, and included the lines listed in Table 1. Plots were harvested at maturity, or at the conclusion of observations.

The isolation method utilized was separation of at least 200 meters from non-transgenic corn not part of the trials and/or detasselling prior to anthesis.

RESULTS

Most lines demonstrated excellent efficacy and no significant negative effect on yield or moisture. Those lines with less efficacy than desired, or having negative agronomic traits associated with the insertion were discontinued from further development. Adequate seed was produced to initiate the next cycle of backcrossing.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Except for the protection against European corn borer (ECB), no disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at the time of harvest in 1994 and is continuing into the 1995 spring season.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 513-11-2, 540-04-1, 544-03-2, 554-03-2,
(PV-ZMBK07+PV-ZMGT10)	557-04-4, 572-16-1, 572-24-1, 575-07-2, 576-01-1,
	581-07-1, 591-03-2, 639-02-1, 654-04-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 600-14-2, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT07	423-06-1
(PV-ZMGT03+PV-ZMGT05)	

[ICI Seeds - Wood River] (Hall County), NE
1994 CORN BELT TRIAL

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-083-03N phenotype: Lepidopteran insect resistance
Monsanto: 94-O89XRA

EXPERIMENT DESCRIPTION

The objective of the plantings was to assess the efficacy of the Btk gene in controlling European Corn Borers (ECB).

The trial was planted May 20, 1994, covering 0.03 acres, and included the lines listed in Table 1. Plots were harvested at maturity, or at the conclusion of observations.

The isolation method utilized was detasselling prior to anthesis.

RESULTS

Most lines demonstrated excellent efficacy against European corn borer (ECB). Those lines with less efficacy than desired, were discontinued from further development.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Except for the protection against ECB, no disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated at harvest in 1994 and is continuing into the 1995 spring season.

Table 1. List of lines planted arranged by vector used in transformation.

Vector	Lines
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	572-16-1, 576-01-1, 581-07-1, 639-02-1, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Blue Earth County, MN

Permit Numbers 94-090XRA, 94-083-04n

Experiment Description Weed Control -- Roundup Ready Corn Field Efficacy

Results
Trial was completed and observations taken as planned.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/11/94 **Harvest/Destroy Date** 8/31/94

Trial Size 0.35 acre

Corn Line Numbers 572-24-1

Field Trial Disposal Method
Trial destroyed and plant material returned to plot area.

Isolation Method Used
Destruction of plot prior to anthesis (flowering).

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

[Northrup King Company - Stanton] (Goodhue County), MN
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-083-04N phenotype: Lepidopteran insect resistance
94-087-12N phenotype: Glyphosate tolerant

Monsanto: 94-O90XRA
94-104XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to introgress the gene of interest (GOI), for either insect protection or glyphosate tolerance, into proprietary inbred lines. Both phenotypes identified above were tested at the same location within the same testing block.

The trial was planted May 26, 1994, covering 0.08 acres, and included the lines listed in Table 1. Plots were harvested at maturity.

The isolation method utilized was separation of at least 200 meters from non-transgenic corn not part of the trials.

RESULTS

Adequate seed was produced to initiate the next cycle of backcrossing.

GENERAL FIELD OBSERVATIONS

There were no unexpected phenotypes. There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Except for the protection against European corn borer (ECB), no disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn or related inbreds and/or hybrids.

DISPOSAL

Following harvest, plant material was disked into the soil.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-159-01N
Pioneer Number:	CORN-HI-94-30N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Cynthia Stauffer 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-3995
Facsimile Telephone Number:	515-222-6883
Date Of This Report:	February 28, 1995

PURPOSE
Seed production.

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site at our research/breeding station located in Kekaha, Kauai, Hawaii. Plantings under this permit were staggered, beginning on July 12, 1994 through November 16, 1994, and comprised approximately 3.26 acres (in total) All open pollinated corn within 660 feet was destroyed. Approximately 65,120 transgenic plants were evaluated for seed production. The grain from all rows was harvested by hand beginning October 6, 1994 and the last harvest occurred on January 25, 1995. The grain was either retained at the secure research facilities in Kekaha, Kauai, for replanting, or shipped to other locations and retained for future plantings. The site remained fallow for at least 30 days.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. Observations of the plants were recorded at different stages during the trials, and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
The plots were cultivated then irrigated to promote germination and any resulting volunteers were destroyed. This cycle was repeated until no volunteers emerged. After approximately 30 days the plots were replanted to corn.

HAWAII OFF-SEASON TESTING AND BREEDING NURSERY
JULY, 1994 PLANTING

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-171-05N phenotype: Lepidopteran insect resistance
94-171-06N phenotype: Glyphosate tolerant

Monsanto: 94-147XRA
94-148XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to produce seed for testing and to introgress the genes of interest (GOI) into elite inbred backgrounds.Both phenotypes identified above were planted into the same nursery block and within the same isolation distance.

Plots were located on Molokai, Hawaii (Maui County). The trial was planted 7/22/94 over an area of 1.87 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested 10/16/94, based on maturity.

The isolation method utilized was at least 200 meter isolation from open-pollinated corn not to be destroyed.

RESULTS

Seed production was as expected under Hawaiian conditions.

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	748-04-3, 754-07-4, 754-10-1
PV-ZMCT01	481-10-1, 540-04-1, 544-03-2, 559-39-2, 572-16-1,
(PV-ZMBK07+PV-ZMGT10)	572-24-1, 574-04-2, 575-07-2, 576-01-1, 581-07-1,
	591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	
PV-ZMCT05	462-03-2
(PV-ZMBK13+PV-ZMGT05)	
PV-ZMCT06	762-03-1
(PV-ZMBK17+PV-ZMGT01)	
PV-ZMCT07	423-06-1
(PV-ZMGT03+PV-ZMGT05)	
V-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

GENERAL FIELD OBSERVATIONS

No disease or insect damage was observed to be more or less severe in transgenic plants than in non-transgenic inbred lines grown as recurrent parents. Rust was the primary disease observed. Insect pressure was primarily from corn ear worms. Some *Bt* lines appeared to have less ear damage than non-*Bt* lines and non-transgenic lines. No characteristics that may lead to increased weediness were noted.

DISPOSAL

Remaining plant material after harvest was disked into the soil.

VOLUNTEERS

Following cultivation, the fields were irrigated, seed allowed to germinate, and resulting volunteer plants destroyed by mechanical or hand cultivation. This cycle was repeated until no volunteer plants were observed.

PUERTO RICO OFF-SEASON TESTING AND BREEDING NURSERY
AUGUST, 1994 PLANTING

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-171-05N phenotype: Lepidopteran insect resistance
94-171-06N phenotype: Glyphosate tolerant

Monsanto: 94-147XRA
94-148XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to produce seed for testing and to introgress the genes of interest (GOI) into elite inbred backgrounds. Both phenotypes identified above were planted into the same nursery block within the same isolation identified below.

The trial was established at the Monsanto Research Farm in Santa Isabel, Puerto Rico, near Ponce. The trial was planted 8/4/94 over an area of 0.17 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested at maturity.

The isolation method utilized was at least 200 meter isolation from open-pollinated corn not to be destroyed.

RESULTS

Seed production was as expected under Puerto Rico conditions.

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FINAL REPORT
Kent Croon

Monsanto Company

Cooperator Name and Address

[CBI DELETED]

Site Location Maui County, HI

Permit Numbers 94-186XRA, 94-265-01n
94-187XRA, 94-265-02n

Experiment Description
Screening and generation advancement of Roundup Resistant and Bt Corn.

Results Trial was completed and observations taken as planned

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No noticeable difference was seen between non-transgenic and transgenic plants. The plot showed the normal disease pressures we would expect from Northern corn leaf blight, Southern corn leaf blight and corn rusts. No difference was seen with respect to nontarget insects including plant hoppers, thrips and mites.

Planting Date 11/9/94 **Harvest/Destroy Date** 2/24/95

Trial Size 0.25 acre

Corn Line Numbers 574-02-1, 574-04-2, 559-52-1, 523-09-1, 540-04-1, 575-26-2, 559-39-2, 544-03-2, 572-04-2, 481-10-1, 591-03-2, 512-03-1, 579-12-1, 575-07-2, 575-30-1, 599-04-3, 627-08-1, 635-11-1, 754-07-4, 462-03-2, 762-03-1, 751-13-1, 694-02-1, 767-07-1, 694-06-1, 768-06-1, 766-07-1, 425-02-1, 425-01-2, 400-01-1, 402-08-1, 423-06-1, 572-16-1, 513-11-2, 523-06-1, 544-04-2, 658-06-1, 546-09-1, 588-13-1, 572-24-1, 581-07-1, 576-01-1, 676-17-1, 639-02-1, 557-04-4, 654-04-1, 554-03-2, 604-09-1, 600-08-3, 635-11-2, 599-04-2, 618-40-1, 600-14-2, 714-05-1, 634-11-1, 631-03-1, 749-01-1, 754-08-1, 754-10-1, 748-04-3.

Field Trial Disposal Method
Trial was harvested and plant material returned to Company location.

Isolation Method Used 200 Meter Isolation

Dates of Monitoring for Volunteer Corn Plants and Number Found
3/25/95, 4/3/95, 4/15/95, 4/24/95 - 10Plt. /Sq. M., 2 Plt./Sq. M., 1 Plt./Sq. M., 0 Plt./Sq.M.

FINAL REPORT
Kent Croon

Monsanto Company

Cooperator Name and Address

[CBI DELETED]

Site Location Maui County, HI

Permit Numbers 94-188XRA, 94-266-01n
94-189XRA, 94-266-02n

Experiment Description
To screen for Bt resistance and to advance this genetic material.

Results Trial was completed and observations taken as planned.

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No noticable difference was seen between non-transgenic and transgenic plants. The plot showed the normal disease pressures we would expect from Northern corn leaf blight, Southern corn leaf blight, and corn rust. No difference was seen with respect to non-target insects including plant hoppers, thrips, and mites.

Planting Date 11/21/94 **Harvest/Destroy Date** 3/15/95

Trial Size 0.12 acre

Corn Line Numbers 574-02-1, 574-04-2, 559-52-1, 523-09-1, 540-04-1, 575-26-2, 559-39-2, 544-03-2, 572-04-2, 481-10-1, 591-03-2, 512-03-1, 579-12-1, 575-07-2, 575-30-1, 599-04-3, 627-08-1, 635-11-1, 754-07-4, 462-03-2, 762-03-1, 751-13-1, 694-02-1, 767-07-1, 694-06-1, 768-06-1, 766-07-1, 425-02-1, 425-01-2, 400-01-1, 402-08-1, 423-06-1, 572-16-1, 513-11-2, 523-06-1, 544-04-2, 658-06-1, 546-09-1, 588-13-1, 572-24-1, 581-07-1, 576-01-1, 676-17-1, 639-02-1, 557-04-4, 654-04-1, 554-03-2, 604-09-1, 600-08-3, 635-11-2, 599-04-2, 618-40-1, 600-14-2, 714-05-1, 634-11-1, 631-03-1, 749-01-1, 754-08-1, 754-10-1, 748-04-3.

Field Trial Disposal Method
Trial was destroyed and plant material returned to plot.

Isolation Method Used
200 meter isolation.

Dates of Monitoring for Volunteer Corn Plants and Number Found
3/29/95–10 plt./sq. M, 4/11/95–10 plt./sq. M, 4/26/95–2 plt./sq. M, 5/12/95–0 plt./sq.M

Method Used to Destroy Volunteers
Volunteers were destroyed as appropriate.

FINAL REPORT

USDA# 94-279-03N, 94-203XRA
USDA# 94-279-04N, 94-204XRA

Gregory B. Parker
Monsanto Co.

Permit Numbers

USDA:	94-279-03N	phenotype: Lepidopteran insect resistance
	94-279-04N	phenotype: Glyphosate tolerant
Monsanto:	94-203XRA	
	94-204XRA	

Experiment Description and Results

The objective of the planting was to produce seed for testing and to introgress the genes of interest into proprietary germplasm. Both phenotypes identified above were planted into the same 200 meter isolation.

Plots were located on Molokai, Hawaii (Maui County). The trial was planted 9/27/95 over an area of 0.12 acres (transgenic). Lines planted included: 599-04-2 (PV-ZMCT02); 631-03-1 (PV-ZMBK10); and 574-04-2, 540-04-1, 481-10-1, 591-03-2, 658-06-1, and 576-01-1(PV-ZMCT01). Harvest was 1/3/96, and plots were destroyed 1/15/96. Generation advancement was successful for all lines.

General Field Observations

Nursery observations indicated no differences in disease or insect damage between transgenic plants and related non-transgenic lines. No characteristics that may lead to increased weediness were observed.

Disposal

Remaining plant material after harvest was disked into soil.

Volunteers

Three observations were made. On 1/29, 24 plants/m2 were observed, and disked 4 days later. On 2/24, 15 plants/m2 were observed, and disked 4 days later. On 3/11, 2 plants/m2 were observed, and disked 11 days later. On the last observation, 4/2, no volunteers were observed. These observations are consistent with those of non-transgenic corn in this environment.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	94-365-03N
Pioneer Number:	CORN-HI-95-01
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 20, 1996

PURPOSE
Winter nursery (off-season testing and breeding)

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered two planting sites (Mahaulepu and Waimea) at our research/breeding station located in Kekeha, Kauai, HW. The trial was planted on staggered dates from 6/21/95 to 7/25/95 (Mahaulepu) and from 2/8/95 to 3/24/95. The Mahaulepu site was 3.25 acres, and the Waimea site was 2.75 acres for a total acreage of 6.0. The trial was isolated by a distance of at least 660 feet from any other corn. Approximately 3250 rows (Mahaulepu) and 2750 rows (Waimea) were evaluated for phenotype. The grain from all rows was harvested by hand on staggered dates from 9/14/95 to 10/23/95 (Mahaulepu) and from 5/23/95 to 6/30/95 (Waimea). The grain from the trial was retained at our facility for future breeding efforts or shipped to our facility in Johnston, IA. Post harvest, the sites remained fallow for at least 30 days.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growingseason. At four stages (approximately every 4 weeks) for each of three planting dates at each of the two sites, the observations were recorded. Results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified plants exhibited altered disease susceptibility. At various time points, light infestations of the following diseases were noted: Northern corn leaf blight, Southern rust, and/or maize dwarf mosaic virus. There were no discernable differences between transgenic and non-transgenic plants.

Light insect feeding was also noted at various time points: aphids, rose beetles, leafhoppers and thrips.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
The plots were cultivated, then irrigated to promote germination. The Mahaulepu site was examined on 1/1/96 and 4/1/96. No volunteer plants were seen. The Waimea site was examined on 8/29/95 and 11/27/95. No volunteer plants were seen.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-032-02N
Pioneer Number:	CORN-NC-95-16N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 19, 1996

PURPOSE
Insect resistance management study

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site near our research/breeding station located in Winterville, Pitt County, North Carolina. The trial was planted on April 14, 1995 (24 rows, 251 plants) and May 3, 1995 (8 rows, 196 plants) and comprised 0.032 acres in total. The trial was isolated by a distance of at least 660 feet from any other corn. The trial was harvested by hand on September 1 and September 22, 1995, respectively. Plant material remaining after harvest was disked into the field.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At two different stages (May 25, 1995 and August 10, 1995) the observations were recorded and the results are listed below.

On May 25, 1995, the transgenic plants were slightly smaller than the non-transgenic plants. By August 10, 1995, the growth differences were no longer discernable. There were no other unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
Remaining vegetative material was destroyed by disking material into the ground on three successive dates until no more volunteers were seen.

POST-TRIAL MONITORING

The following season tobacco was planted. On July 1, 1996, the site was monitored for volunteer corn and no volunteers were found.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-037-07N
Pioneer Number:	CORN-KY-95-19N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 19, 1996

PURPOSE
Insect resistance management study

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting near Lexington, Fayette County, Kentucky. The trial was planted on May 8, 1995 and comprised approximately 0.07 acres. The trial was isolated by a distance of at least 660 feet from any other corn. Transgenic corn was observed for insect refugia and natural enemies of insect pests. No seed was harvested or retained. The plots were cut/chopped with a rotary mower and the debris was plowed several inches under the soil surface with a moldboard plow on November 16, 1995.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and entomology during the growing season. No unusual observations were reported.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All vegetative material was chopped and returned to the plot for soil composting on November 16, 1995.

POST-TRIAL MONITORING

The plot area was disked during the spring of 1996 and soybeans were planted on the entire area on May 20, 1996. The area was monitored for volunteer corn. On June 19, 1996, 23 volunteers were observed and destroyed by hand weeding. On July 26, 1996, no volunteers were observed.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-039-09N
Pioneer Number:	CORN-MO-95-20N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 19, 1996

PURPOSE
Breeding and observation nursery, and line *per se* yield trial

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site (Miami, Saline County, MO) near our research/breeding station located in Carrollton, Carroll County, MO. The trial was planted on June 12, 1995 and comprised 0.84 acres. The trial was isolated by a distance of at least 660 feet from any other corn. Approximately 23, 500 plants of transgenic corn were evaluated for phenotype and insect resistance. On October 9, 1995, the grain was harvested, shelled, and cultivated in to decompose. None was retained.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At one stage (September 4, 1995) the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepitopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
The site was examined on August 21, 1996 for volunteer corn. No volunteer plants were observed.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-039-11N
Pioneer Number:	CORN-SD-22N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 19, 1996

PURPOSE
Gene efficacy trial

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site (Cavour, Beadle County) near our research/breeding station located in Huron, Beadle County, SD. The trial was planted on May 17,1995 and comprised 0.17 acres. The trial was isolated by a distance of at least 660 feet from any other corn. Approximately 4200 plants were evaluated for insect resistance. The grain from all rows was harvested by machine around October 3, 1995. All grain was destroyed and none was retained..

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At two different stages (July 13 and August 17, 1995) the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
The following season the area was planted with transgenic, herbicide-resistant corn. Herbicide application and hand weeding used to remove volunteers.
5/28/96: no volunteers were observed
6/28/96: five volunteer plants were observed and destroyed
7/29/96: no volunteers were observed

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-039-12N
Pioneer Number:	CORN-WI-95-23N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 20, 1996

PURPOSE
Line per se yield trial
Breeding and observation nursery
Efficacy demonstration

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered two planting sites at our research/breeding station located in Janesville, Rock County, WI. The sites were planted on May 4 and May 19, 1995 and comprised 1.25 acres. The trial was isolated by a distance of at least 660 feet from any other corn. Transgenic plants were evaluated for phenotype and insect resistance. The grain from all rows was harvested by machine on September 30, 1995. All grain was destroyed and none was retained.. In the spring of 1996, the site was replanted to soybeans.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At two different stages (July 10 and September 30, 1995) the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
The sites were examined on October 2, 1995 for volunteer plants. None were observed. On July 10, 1996, 17 volunteer plants were observed. They were destroyed with herbicide treatment.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-039-13N
Pioneer Number:	CORN-MI-95-24N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 19, 1996

PURPOSE
Breeding and observation nursery

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site at Breckenridge near our research/breeding station located in Ithaca,Gratiot County, MI. The trial was planted on May 8, 1995 and comprised 0.53 acres (26,000 plants/acre). The grain from all rows was harvested by machine on approximately September 25, 1995. All grain was destroyed and none was retained. In the spring of 1996, the site was replanted with soybeans.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At three different stages (June 15, July 21 and September 14, 1995) the observations were recorded, and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING

The site was monitored for volunteers on August 19, 1996. Less than 50 plants were observed, and these were destroyed by hand weeding and mechanical cultivation.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-039-14N
Pioneer Number:	CORN-MN-95-25N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 19, 1996

PURPOSE
Breeding and observation nursery (Mankato and Morehead sites) and line *per se* yield trial (Mankato site)

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered two planting sites at our research/breeding stations located in Mankato, Blue Earth County, MN and Morehead, Clay County, MN. The Morehead site (0.24 acres) was planted on May 22, 1995 and the Mankato site (0.5 acres) was planted on May 18, 1995. The trial was isolated by a distance of at least 660 feet from any other corn. Transgenic corn was evaluated for phenotype and agronomic effects of the inserted genes. At Morehead, the grain from all rows was harvested by hand on September 15, 1995. Some was shipped to our facility in Kauai, HW and the remainder was shipped to our facility in Johnston, IA. At Mankato, because of excessive rainfall causing drowned and/or variable plants, the plot was destroyed on August 10, 1995 by mechanical cultivation. This was well before physiological maturity (viable seed set) had occurred. In the spring of 1996, the Morehead site was replanted to a crop other than corn, and the Mankato site was replanted to corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At several different stages the observations were recorded and the results are listed below. The Morehead site was observed June 26, July 19 and September 15, 1995. The Mankato site was observed on July 6, 1995.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
The Morehead site was monitored for volunteer plants on October 17, 1995 and May 21, June 19 and August 19, 1996. Three volunteer plants were observed on June 19, 1996 and the plants were hand destroyed.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-039-15N
Pioneer Number:	CORN-IN-95-27N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 20, 1996

PURPOSE
Breeding and observation nursery and line *per se* yield trial

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered three planting sites near our research/breeding station located in Windfall, Tipton County, IN. Two of the sites were in Tipton County, and the other was in Wells County. The Tipton County sites were planted on May 11, 1995, and comprised 0.764 acres in total. The Wells County site was planted May 22, 1995 and comprised 0.48 acres. The plants were observed for phenotype and agronomic effects of the inserted genes. The grain from all rows was harvested by machine on September 15, 1995. All grain was destroyed and none was retained.. In the spring of 1996, the sites were replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At four different stages (June 9, July 7, August 3 and September 7, 1995) the observations were recorded and the results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms which would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
Tipton County site #1 was checked for volunteer plants on May 30, June 22, and June 27, 1996. No volunteers were observed.
Tipton County site #2 was checked for volunteer plants on May 30, June 19 and June 28, 1996. No volunteers were observed.
The Wells County site was checked for volunteer plants on May 21, June 11 June 26, 1996. No volunteers were observed.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-039-16N
Pioneer Number:	CORN-NE-95-28N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 20, 1996

PURPOSE
Gene efficacy
Breeding and observation nursery

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered one planting site at our research/breeding station located in York, York County, NE. The trial was planted on May 17, 1995 and comprised 0.226 acres. The trial was isolated by a distance of at least 660 feet from any other corn. 4695 plants of transgenic corn were evaluated for phenotype and insect resistance. The grain from all rows was harvested by machine around October 9, 1995. All grain was destroyed and none was retained. In the spring of 1995, the site was replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At two different stages (July 12 and August 14, 1995) the observations were recorded. The results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
On 8/21/96, the site was monitored for volunteer plants. None were observed.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	95-074-11N
Pioneer Number:	CORN-IA-95-30N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 11252 Aurora Avenue Des Moines, IA 50322
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	August 20, 1996

PURPOSE
Gene efficacy trial
Breeding and observation nursery
Line *per se* yield trial
Roundup tolerance yield trial

SUMMARY OF EXPERIMENTAL RESULTS
This permit covered ten planting sites near our research/breeding stations.

Sites	plant date	rows/acres	harvest date
Algona, Kossuth County, IA	5/6/95	0.448 acres/9,800 plants	10/7/95
Callendar, Webster County, IA	5/18/95	0.426 acres/10,200 plants	10/7/95
Woodward, Boone County, IA	5/25/95	2.5 acres	10/2-3/95
Martensdale, Warren County, IA	5/20/95	1.5 acres	10/2-3/95
Sheldahl #1, Polk County, IA	5/30/95	25,900 plants/1.036 acres	9/23/95
Sheldahl #2, Polk County, IA	5/25/95	16 rows/0.016 acres	9/23/95
Johnston #1, Polk County, IA	5/14/95	43,610 plants/1.744 acres	9/23/95
Johnston #2, Polk County, IA	4/25/95	16 rows/0.016 acres	9/23/95
Marion, Linn County, IA	5/17/95	1.456 acres	10/9/95
Vinton, Benton County, IA	5/17/96	0.738 acres	10/10/95

The trial was isolated by a distance of at least 660 feet from any other corn. The grain from all rows was harvested by machine. All grain was destroyed and none was retained. In the spring of 1995, the sites were replanted to a crop other than corn.

GENERAL FIELD OBSERVATIONS
The plants were frequently observed by personnel experienced in corn breeding and plant pathology during the growing season. At several different stages the observations were recorded, and the dates and results are listed below.

Algona, Kossuth County, IA	6/16/95	6/30/95	8/39/95	9/13/95
Callendar, Webster County, IA	6/20/95	7/11/95	8/16/95	
Woodward, Boone County, IA	6/19/95	7/21/95	8/11/95	
Martensdale, Warren County, IA	6/20/95	7/18/95	8/8/95	
Sheldahl #1, Polk County, IA	6/30/95	7/30/95	8/31/95	
Sheldahl #2, Polk County, IA	6/30/95	7/30/95	8/31/95	
Johnston #1, Polk County, IA	6/30/95	7/30/95	8/31/95	
Johnston #2, Polk County, IA	6/30/95	7/30/95	8/18/95	
Marion, Linn County, IA	6/27/95	8/7/95	8/29/95	
Vinton, Benton County, IA	5/17/95	7/24/95	8/4/95	8/18/95

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There was no evidence that the modified organism exhibited altered disease susceptibility. Gray leaf spot, eye spot and northern leaf blight were observed at Algona on 9/13/95. Gray leaf spot was observed at Woodward on 8/11/95, at Martensdale on 8/8/95, at Marion on 8/7/95, and at Vinton on 8/4/95. There was no apparent differences in disease susceptibility between transgenics and non-transgenics.

The only observation of altered pest susceptibility was lepidopteran resistance, which is the trait that the plants had been modified to exhibit.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was chopped and returned to the plot for soil composting.

POST-TRIAL MONITORING
Sites were monitored the following year for volunteer corn.

Algona, Kossuth County	6/15/96	6 volunteers observed and destroyed by hand weeding
	7/15/96	cooperator used herbicide treatment
	8/21/96	3 volunteers observed and destroyed by hand weeding
Callendar, Webster County	6/15/96	cooperator used herbicide treatment and mechanical cultivation
	7/8/96	10 volunteers observed and destroyed by hand weeding
	8/21/96	23 volunteers observed and destroyed by hand weeding
Woodward, Boone County	7/15/96	no volunteers were observed
Martensdale, Warren County	7/15/96	<50 volunteers were observed and removed by hand weeding
Sheldahl #1, Polk County	6/15/96	volunteers had been destroyed with herbicide none were seen on subsequent checks
Sheldahl #2, Polk County	6/15/96	volunteers had been destroyed with herbicide none were seen on subsequent checks
Johnston #1, Polk County	6/15/96	volunteers had been destroyed with herbicide none were seen on subsequent checks
Johnston #2, Polk County	6/15/96	volunteers had been destroyed with herbicide none were seen on subsequent checks
Marion, Linn County	7/19/96 8/2/96 8/20/96	Cooperator had planted corn into the edges of plot. This corn was mowed down on July 19, 1996, prior to any pollen shed. Volunteers within the plot area were also removed July 19. The field was re-checked on August 2 and August 20, with additional volunteers removed on those dates by hoeing and mowing with rotary mower, again prior to pollen shed of the volunteers.
Vinton, Benton County	7/29/96	no volunteers were observed

FINAL REPORT

USDA# 95-072-03n/95-091XRA
USDA# 95-072-04n/95-092XRA

Gregory B. Parker
Monsanto Co.

Permit Numbers

USDA: 95-072-03N phenotype: Lepidopteran insect resistance
95-072-04N phenotype: GIyphosate tolerant

Monsanto: 95-091XRA
95-092XRA

Experiment Description and Results

Two types of trials were planted under these notifications: two gene efficacy studies, and three nurseries

Gene Efficacy Studies

Glyphosate Tolerance

The objective of this trial was to assess the tolerance of corn to various rates and timing of Roundup™ herbicide. This trial was planted at Waterloo, Nebraska in Douglas County on 6/13/96, and transgenic plants covered an area of 1.00 acres. Both phenotypes listed above were planted into the same experimental block within the same 200 meter isolation distance. Lines planted included 599-04-2 (PV-ZMCT02), 631-03-1 (PV-ZMBK10), and 574-04-2 and 591-03-2 (PV-ZMCT01). Crop destruction occurred 9/25/95. Late planting coupled with extreme heat and drought precluded collection of meaningful data from this experiment.

European Corn Borer (ECB) Resistance

The objective of this trial was to assess the reaction of several transgenic lines of corn to natural and artificial infestations of ECB. This trial was planted at Clinton, Illinois in DeWitt County on 5/31/96, and transgenic plants covered an area of 0.12 acres. Both phenotypes listed above were planted into the same experimental block within the same 200 meter isolation distance. Lines planted included: 599-04-2 (PV-ZMCT02); 631-03-1 (PV-ZMBKIO); 481-10-1, 591-03-2, 658-06-1, and 576-01-I (PV-ZMCTO1); 958-122 and 997-21-5 (PV-ZMBKIOL); 1032-06-14 and 1032-19-14 (PV-ZMBK20L); and 992-09-1 (PV-ZMBK12L). Harvest was *10/2/95,* and plots were destroyed 10/10/95. Lines varied with respect to protection from ECB. Those with the best resistance were continued in breeding programs.

Nurseries

The objectives of these nurseries were to introgress genes of interest into proprietary germplasm. There were three separate nurseries; one in Ray County, Missouri, near Henrietta, one in DeWitt County, Illinois, near Clinton, and the third in LaFayette County, Wisconsin, near Platteville. In each nursery, both phenotypes listed above were planted into the same nursery block.

DeWitt County, IL

Plots were planted May 23, 1995. Transgenic acreage was 0.128 acres. Lines planted included: 599-04-2 and 600-14-2 (PV-ZMCT02); 631-03-1 (PV-ZMBK10); 481-10-1, 591-032, 658-06-1, 574-04-2, 654-04-1, and 576-01-I (PV-ZMCTO1); 958-12-2 and 997-21-5 (PVZMBKIOL); 1032-06-14 and 1032-19-14 (PV-ZMBK20L); and 992-09-1 (PV-ZMBKI2L). Both phenotypes listed above were planted into the same experimental block within the same .200 meter isolation distance, and transgenic plants were detasseled. Harvest was 9/28/95, and plots were destroyed 10/6/95. Generation advancement was successful for all lines.

LaFayette County, WI

Plots were planted May 4, 1995. Transgenic acreage was 0.134 acres. Lines planted included: 599-04-2 (PV-ZMCT02); 631-03-1 (PV-ZMBKIO); 574-04-2, 540-04-1,481-10-1, 591-03-2, 658-06-1, and 576-01-I (PV-ZMCTO1); 958-12-2 and 997-21-5 (PV-ZMBKIOL); 1032-06-14 and 1032-19-14 (PV-ZMBK20L); and 992-09-1 (PV-ZMBKI2L). Both phenotypes listed above were planted into the same experimental block within the same 100 meter isolation distance, and all transgenic plants were detasseled. Harvest and crop destruction was on 11/1/95. Generation advancement was successful for all lines.

Ray County, MO

Plots were planted June 14, 1995. Transgenic acreage was 0.307 acres. Lines planted included: 599-04-2 (PV-ZMCT02); 631-03-1 (PV-ZMBKIO); 574-04-2, 481-10-1, 591-03-2, 658-06-1, and 576-01-I(PV-ZMCT01); 958-12-2 and 997-21-5 (PV-ZMBK10L); 1032-06-14 and 1032-19-14 (PV-ZMBK20L); and 992-09-1 (PV-ZMBKI2L). All transgenic plants were detasseled or had pollination bags placed on them during the course of anthesis. Harvest and crop destruction was on 10/7/95. Generation advancement was successful for all lines.

General Field Observations

Observation in all the experiments described above indicated no differences in disease or insect damage between transgenic plants and in related non-transgenic lines, except plants with Bt showed tolerance to ECB and some Lepidopteran insects. No characteristics that may lead to increased weediness were observed.

Disposal

Remaining plant material after harvest was disked into soil.

Volunteers

At the Dewitt County, LaFayette County, and Ray County sites, no volunteers were observed in the fall after crop destruction, nor during the following spring. At the Douglas County site, approximately 200,000 volunteer plants were estimated to be present on June 10, prior to cultivation and soybean seeding. The area encompassed both transgenic and non-transgenic plantings of the previous year. No differences were observed in volunteer plant density or distribution where the transgenic plots had been.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Stafford County KS

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Determine efficacy of Bt corn against European Corn borer, Southwestern Corn borer and corn earworm.

Results
Mon 810 held up to heavy Southwestern corn borer pressure compared to the susceptible line. Preliminary data indicate no difference in corn earworm presence between the transgenic lines and control line.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/11/95 and 5/19/95 **Harvest/Destroy Date** 10/5/95

Trial Size 0.44 acre

Corn Line Numbers 599-04-2, 658-06-1.

Field Trial Disposal Method
Trial destroyed and plant material returned to plot area.

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Tippecanoe County, IN

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description Evaluate Bt efficiency.

Results
Excellent weed control, below average yield due to severe drought.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 4/28/95 **Harvest/Destroy Date** 10/13/95

Trial Size 0.165 acre

Corn Line Numbers 599-04-2, 658-06-1, 631-03-1, 576-01-1

Field Trial Disposal Method Burning

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Riley County KS

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Determine levels of Bt expression in key corn tissue and to determine efficacy of Bt corn against European corn borer and corn earworm.

Results
Transgenic corn performed well against European corn borer compare to the control line. Preliminary data indicate no difference in corn earworm presence between the transgenic and control lines.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/15/95 and 5/23/95 **Harvest/Destroy Date** 10/20/95

Trial Size 0.40 acre

Corn Line Numbers 599-04-2, 658-06-1, 631-03-1, 576-01-1

Field Trial Disposal Method
Trial was destroyed and plant material returned to plot area.

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Scott County, IN

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Evaluate insect resistance and yield responses to feeding of European corn borer.

Results
Missed early flight of ECB because of late planting. Did have some late generation feeding and boring. Mon 802 appeared to have the fewest plants affected and least tunneling. No yield taken due to poor stand achieved.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 6/9/95 **Harvest/Destroy Date** 10/29/95

Trial Size 0.14 acre

Corn Line Numbers 599-04-2, 658-06-1, 631-03-1, 576-01-1

Field Trial Disposal Method Mowing (Bush-hog)

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Story County, IA

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Compare yield and performance of two genetically modified lines of corn with appropriate controls, and compare Bt expression in four genetically modified lines.

Results
Mon 810 and Mon 802 were effective in controlling ECB damage. Both yielded better than non-transgenic controls.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/22/95 **Harvest/Destroy Date** 12/6/95

Trial Size 0.4 acre

Corn Line Numbers 599-04-2, 658-06-1, 631-03-1, 576-01-1

Field Trial Disposal Method Disking

Isolation Method Used
Greater than 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Story County, IA

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Compare susceptibility of genetically modified line of corn with appropriate control to *Beauveria Bassiana*

Results
Beauveria Bassiana formed an endonytic relationship equally in Bt transgenic plants and non-transgenic plants

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/25/95 **Harvest/Destroy Date** 10/19/95

Trial Size 0.4 acre

Corn Line Numbers 599-04-2

Field Trial Disposal Method Disking

Isolation Method Used Greater than 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Madison County, IL

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description Evaluate insect efficacy and yield of Bt corn.

Results Trial not harvested.

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 6/7/95 **Harvest/Destroy Date** 10/10/95

Trial Size 0.25 acre

Corn Line Numbers 658-06-1, 576-01-1, 599-04-2, 631-03-1.

Field Trial Disposal Method Entire area moved to destroy

Isolation Method Used 660 feet isolation

Comments Trial not harvested

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Kent County, MD

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Examine the yield advantage of Bt corn lines under natural insect pressure.

Results
Bt positive lines gave protection from ECB.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/23/95 **Harvest/Destroy Date** 10/4/95

Trial Size 0.25 acre

Corn Line Numbers 599-04-2, 658-06-1, 576-01-1, 631-03-1.

Field Trial Disposal Method
Plants scattered on ground and disked. Frost killed vlunteers.

Isolation Method Used
200 Meter Isolation.

Dates of Monitoring for Volunteer Corn Plants
7/11/96 - None

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Story County, IA

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Evaluation of stalk borer, armyworm and corn earworm feeding and damage on transgenic Bt corn

Results
Trial was completed and observations taken as planned. The number of predators were counted on Mon 810+ and Mon 810- plants at three different times near anthesis: August 1st (before pollen shed), August 6th (during pollen shed), and August 18th (after pollen shed). Eighteen plants were marked; six per replication, within each treatment. The same plants were checked on each sampling date. The predators present consisted of coccinellid eggs, larvae, and adults, chrysopid eggs, larvae, and adults, anthocorid nymphs and adults, nabids and arachnids. The number of ECB egg masses were also recorded. Differences were observed in abundance of predators at each sampling time, but no differences were found between the corn types (Mon 810+and Mon 810-).

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn.

Planting Date 5/24/95 **Harvest/Destroy Date** 10/19/95

Trial Size 0.069 acre

Corn Line Numbers 658-06-1

Field Trial Disposal Method Disking

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Champaign County, IL

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
1) To evaluate the insect resistance of the leading Bt and Bt/RR corn events. 2) To evaluate Bt-corn and control corn yield responses to feeding injury from the ECB. 3) To compare the insect resistance of Bt-corn to untreated control corn or control corn treated with a standard commercial insecticide program. 4) to examine Bt-corn effects on other lepidoptera.

Results
Trial was completed and observations showed no obvious differences between the control and transgenic corn other than control of European corn borer.

General Field Observations There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. Smut, flea beetles, Hippodamia, Coleomegilla, Lacewings, grasshoppers and nitidulids were observed, however there were not differences noted between transgenic and non-transgenic plants.

Planting Date 6/7/95 **Harvest/Destroy Date** 9/26/95

Trial Size 0.5 acre

Corn Line Numbers 658-06-1, 599-04-2.

Field Trial Disposal Method
Corn stalks were retained standing for future stalk-splitting in the spring of 1996 and then destroyed.

Isolation Method Used
262 Meter Isolation.

Dates of Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Knox County, IL

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Verify levels of Bt expression throughout the entire plant through the whole season.

Planting Date 5/22/95

Trial Size 0.009 acre

Corn Line Numbers 658-06-1, 576-01-1, 599-04-2, 631-03-1.

Isolation Method Used 200 Meter Isolation.

Comments
Trial was destroyed by cattle on July 20, 1995. Cooperator immediately notified Monsanto, and Monsanto as the U.S.D.A. Notification applicant immediately informed the appropriate regulatory agencies with written correspondence.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Finney County, KS

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Evaluate the insect resistance of the leading Bt corn events and yield evaluation.
Results
Trial was completed and observations taken as planned.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn. Some temporary plant yellowing due to herbicides and cool weather, however there were no differences noted between transgenic and non-transgenic plants.

Planting Date 5/11/95 **Harvest/Destroy Date** 11/1/95

Trial Size 0.248 acre

Corn Line Numbers 599-04-2, 658-06-1.

Field Trial Disposal Method
Plants cut with silage cutter-dumped-burned with harvested seed.

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Finney County, KS

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Bt gene expression: Genotype by environment effects

Results
Trial was completed and observations taken as planned.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn. Some temporary plant yellowing due to herbicides and cool weather, however there were no differences noted between transgenic and non-transgenic plants.

Planting Date 5/11/95 **Harvest/Destroy Date** 11/1/95

Trial Size 0.0046 acre

Corn Line Numbers 599-04-2, 658-06-1, 576-01-1, 631-03-1.

Field Trial Disposal Method
Plants cut with silage cutter-dumped-burned with harvested seed.

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Finney County, KS

Permit Numbers 95-100XRA, 95-075-01n

Experiment Description
Efficacy of Bt corn in reducing kernel damage by corn earworms.

Results
Trial was completed and observations taken as planned.

General Field Observations
There was no evidence to suggest that these transgenic plants would be any more or less weedy than traditional dent corn. No disease or insect damage was observed to be more or less severe in transgenic plants than in typical non-transgenic corn. Some temporary plant yellowing due to herbicides and cool weather, however there were no differences noted between transgenic and non-transgenic plants.

Planting Date 5/11/95 **Harvest/Destroy Date** 11/1/95

Trial Size 0.044 acre

Corn Line Numbers 599-04-2, 658-06-1.

Field Trial Disposal Method
Plants cut with silage cutter-dumped-burned with harvested seed.

Isolation Method Used 200 Meter Isolation.

Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Benton County, IA

Permit Numbers 95-116XRA, 95-083-10n
95-117XRA, 95-083-11n

Experiment Description
The production plant tissues and grain to support regulatory approvals of Bt and Roundup Ready corn lines expressing the CryIA(b), CP4 EPSPS, and/or GOX proteins.

Results
Corn plants were produced under Good Laboratory Practices (GLP) and plant tissues were collected and shipped for analyses at varying times in the plant growth cycle.

General Field Observations
Corn plant growth appeared normal with no unusual observations in terms of potential weediness or disease or insect susceptibility. Tolerance to feeding of European corn borer was observed in corn lines expressing the CryIA(b) protein.

Planting Date 5/26/95 **Harvest/Destroy Date** 10/6/95

Trial Size 0.050 acre

Corn Line Numbers
599-04-2, 631-03-1, 572-16-1, 658-06-1, 600-14-2, 654-04-1, 481-10-1, 574-04-2, 591-03-2.

Field Trial Disposal Method
Plot area was destroyed through tillage and plant material returned to the plot area.

Isolation Method Used
200 Meter Isolation.

Dates of Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Jersey County, IL

Permit Numbers 95-116XRA, 95-083-10n
95-117XRA, 95-083-11n

Experiment Description
The production plant tissues and grain to support regulatory approvals of Bt and Roundup Ready corn lines expressing the CryIA(b), CP4 EPSPS, and/or GOX proteins.

Results
Corn plants were produced under Good Laboratory Practices (GLP) and plant tissues were collected and shipped for analyses at varying times in the plant growth cycle.

General Field Observations
Corn plant growth appeared normal with no unusual observations in terms of potential weediness or disease or insect susceptibility. Tolerance to feeding of European corn borer was observed in corn lines expressing the CryIA(b) protein.

Planting Date 5/23/95 **Harvest/Destroy Date** 10/13/95

Trial Size 0.7 acre

Corn Line Numbers 599-04-2, 631-03-1, 572-16-1, 658-06-1, 600-14-2, 654-04-1, 481-10-1, 574-04-2, 591-03-2

Field Trial Disposal Method
Plot area was destroyed through tillage and plant material returned in to the plot area.

Isolation Method Used
Plants were bagged before and during anthesis.

Dates of Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Saunders County, NE

Permit Numbers 95-116XRA, 95-083-10n
95-117XRA, 95-083-11n

Experiment Description
The production plant tissues and grain to support regulatory approvals of Bt and Roundup Ready corn lines expressing the CryIA(b), CP4 EPSPS, and/or GOX proteins.

Results
Corn plants were produced under Good Laboratory Practices (GLP) and plant tissues were collected and shipped for analyses at varying times in the plant growth cycle.

General Field Observations
Corn plant growth appeared normal with no unusual observations in terms of potential weediness or disease or insect susceptibility. Tolerance to feeding of European corn borer was observed in corn lines expressing the CryIA(b) protein.

Planting Date 5/25/95 **Harvest/Destroy Date** 10/11/95

Trial Size 0.02 acre

Corn Line Numbers 599-04-2, 631-03-1, 572-16-1, 658-06-1, 600-14-2, 654-04-1, 481-10-1, 574-04-2, 591-03-2

Field Trial Disposal Method
Plot area was destroyed through tillage and plant material returned to the plot area.

Isolation Method Used 200 Meter Isolation.

Dates of Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Tippecanoe County, IN

Permit Numbers 95-116XRA, 95-083-10n
95-117XRA, 95-083-11n

Experiment Description
The production plant tissues and grain to support regulatory approvals of Bt and Roundup Ready corn lines expressing the CryIA(b), CP4 EPSPS, and/or GOX proteins.

Planting Date 6/1/95

Trial Size 0.02 acre

Corn Line Numbers
599-04-2, 631-03-1, 481-10-1, 574-04-2, 591-03-2

Comments
Experiment was discontinued due to poor growing conditions and plot area was destroyed.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Warren County, IL

Permit Numbers 95-116XRA, 95-083-10n
95-117XRA, 95-083-11n

Experiment Description
The production plant tissues and grain to support regulatory approvals of Bt and Roundup Ready corn lines expressing the CryIA(b), CP4 EPSPS, and/or GOX proteins.

Results
Corn plants were produced under Good Laboratory Practices (GLP) and plant tissues were collected and shipped for analyses at varying times in the plant growth cycle.

General Field Observations
Corn plant growth appeared normal with no unusual observations in terms of potential weediness or disease or insect susceptibility. Tolerance to feeding of European corn borer was observed in corn lines expressing the CryIA(b) protein.

Planting Date 6/2/95 **Harvest/Destroy Date** 10/18/95

Trial Size 0.05 acre

Corn Line Numbers
599-04-2, 631-03-1, 572-16-1, 600-14-2, 654-04-1, 481-10-1, 574-04-2, 591-03-2.

Field Trial Disposal Method
Plot area was destroyed through tillage and plant material returned to the plot area.

Isolation Method Used 200 Meter Isolation.

Dates of Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

FINAL REPORT
Kent Croon

Monsanto Company

Site Location Story County, IA

Permit Numbers 95-116XRA, 95-083-10n
95-117XRA, 95-083-11n

Experiment Description
The production plant tissues and grain to support regulatory approvals of Bt and Roundup Ready corn lines expressing the CryIA(b), CP4 EPSPS, and/or GOX proteins.

Results
Corn plants were produced under Good Laboratory Practices (GLP) and plant tissues were collected and shipped for analyses at varying times in the plant growth cycle.

General Field Observations
Corn plant growth appeared normal with no unusual observations in terms of potential weediness or disease or insect susceptibility. Tolerance to feeding of European corn borer was observed in corn lines expressing the CryIA(b) protein.

Planting Date 5/26/95 **Harvest/Destroy Date** 10/6/95

Trial Size 0.019 acre

Corn Line Numbers
599-04-2, 631-03-1, 572-16-1, 658-06-1, 600-14-2, 654-04-1, 481-10-1, 574-04-2, 591-03-2

Field Trial Disposal Method
Plot area was destroyed through tillage and plant material returned to the plot area.

Isolation Method Used
Shoots and tassels bagged.

Dates of Monitoring for Volunteer Corn Plants
Monitoring was conducted and volunteers were destroyed as appropriate.

JERSEYVILLE, ILLINOIS FIELD TRIALS
1995 GROWING SEASON

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-321-03N, 95-080-05N, 95-087-10N, 95-087-09N, 95-096-08N
MONSANTO: 94-228XRA, 95-102XRA, 95-120XRA, 95-121XRA, 95-134XRA

EXPERIMENT DESCRIPTIONS

Several trials were conducted under these notifications. Objectives included determination of the efficacy of the gene of interest and associated seed increases and gene introgression into elite inbred lines(GOI)(AG-Mon-95-04), assessments of effects of the inserted gene on agronomic traits (AG-CT-95-02, AG-Mon-95-06), and evaluation of the protection afforded by gene combinations (AG-Mon-95-01).

The trials were established at the Monsanto Research Farm in Jerseyville, Illinois, in Jersey County. The transgenic lines planted in these trials are listed in Table 1. It should be noted that line 1171-21-7, of vector PV-ZMBK21L, was unintentionally omitted from the list of lines that were to be field tested in the original notification and subsequent modification. Trials were planted over the period May 12 - June 5, 1995, and harvested at maturity. Plots were isolated at least 200 meters from any other corn not part of these trials that was allowed to go to flower. Total area of the trials was 9.8 acres, of which 4.2 were transgenic.

RESULTS

For previously tested lines, efficacy was confirmed in most cases; those lines not demonstrating adequate protection were discontinued. Similarly, lines evidencing agronomic deficiencies such as lower yield or higher moisture attributable to the GOI were discontinued from further developments. New lines were compared to previously selected lines for efficacy. Several lines were identified that had similar efficacy, and will be evaluated again. Seed production was excellent, and comparable to non-transgenic versions of the particular recurrent parent.

GENERAL FIELD OBSERVATIONS

Transgenic lines were compared to either related non-transgenic inbreds or hybrids or to sibs that did not have the GOI. No differences were noted in disease or insect susceptibility. No line exhibited any characteristic that might lead to increased weediness, such as excessive tillering, seed shattering, or a perennial habit.

FIELD TRIAL DISPOSAL METHOD

Following harvest, plots were chopped and residue incorporated into the soil.

VOLUNTEERS

Monitoring for volunteers has been completed and field sites have been rotated to alternate crops.

Table 1. List of transgenic lines tested in Jerseyville, IL, during the 1995 growing season, arranged by vector used in the transformation

PV-ZMBK10
631-03-1

PV-ZMBK10L

958-12-2	997-21-5

PV-ZMBK12L

749-01-1	992-09-1

PV-ZMBK16L
748-04-3

PV-ZMBK20L

1032-06-14	1032-19-14

PV-ZMBK21L

1171-21-7	1208-12-04	1208-17-01	1325-1a-14	1325-1a-17
1325-2a-10	1325-2a-2	1325-2a-23	1325-3b-11	1325-3b-7
1325-4a-30	1325-4a-33	1325-4a-35	1325-4a-4	1325-4b-14
1325-4b-23	1325-4b-9	1325-5-2	1326-01-04	1326-05-01
1327-02-07	1329-01-03	1329-04-04	1329-04-06	1329-04-11
1329-08-02	1329-08-03	1331-01-01	1335-01-19	1367-21-06
1367-22-02	1367-23-07	1367-24-07	1367-25-13	1378-03-06
1392-01-07	1392-01-13	1392-01-25	1392-01-26	1392-01-29
1392-01-40	1392-02-04	1392-02-12	1392-02-18	1392-02-19
1392-02-29	1392-03-16	1392-03-20	1392-03-32	1392-03-51
1392-03-54	1392-04-03	1392-04-04	1392-04-19	1392-04-29
1392-04-39	1392-04-41	1392-04-44	1392-04-48	1392-05-02
1392-05-07	1392-05-27	1392-06-15	1411-04-15	1411-12-14
1411-14-15	1411-14-17			

PV-ZMGT18L

1112-02-1	1163-07-2

PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)

481-10-1	540-04-1	559-39-2	572-16-1	574-04-2
575-07-2	576-01-1	591-03-2	654-04-1	658-06-1

USDA: 94-321-03N, 95-080-05N, 95-087-10N, 95-087-09N, 95-096-08N
MONSANTO: 94-228XRA, 95-102XRA, 95-120XRA, 95-121XRA, 95-134XRA

PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)
599-04-2 599-04-3 600-14-2 604-09-1

PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)
462-03-2

PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)
423-06-1

PV-ZMMT01 (PV-ZMBK25+PV-ZMSM06+PV-ZMSM10)
236-08-10

PV-ZMIR01
1342-07-01 1348-11-01