95-093-01p

Monsanto

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March 30, 1995

Mr. Michael A. Lidsky
Deputy Director, BBEP, APHIS, USDA
Att: BCPA
4700 River Road
Unit 146
Riverdale, MD 20737-1237

Subject: Petition for Determination of Non-Regulated Status: Insect Protected Corn Line MON 80100.
Monsanto #: 95-119U

Dear Mr. Lidsky:

The Agricultural Group of Monsanto Company is submitting a Petition for Determination of Non-Regulated Status to the Animal and Plant Health Inspection Service (APHIS) regarding Insect Protected Corn line MON 80100. This petition requests a determination from APHIS that the line MON 80100 and any progenies derived from crosses between line MON 80100 and traditional corn varieties no longer be considered a regulated article under regulations in 7 CFR part 340. Insect protected corn line MON 80100 has been tested for 3 years at 62 locations in thirteen states. The copies of the final reports for these field trials are included in this petition.

We appreciate your attention to this matter. Should you have any questions, please feel free to contact either Dr. Dickerson at 202-783-2460 or myself (314-537-7488).

Sincerely,

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

cc: Dr. C.T. Dickerson - Monsanto

#-1 Ck 4/3/1

Petition for Determination of Nonregulated Status:

Insect Protected Corn (*Zea mays* L.) with the *cryIA(b)* Gene from *Bacillus thuringiensis* subsp. *kurstaki*

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

Submitted by:

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March 30, 1995
#95-119U

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Contains Confidential Business Information

Petition For Determination of Nonregulated Status for Insect Protected Corn (*Zea mays* L.) with the *cryIA(b)* gene from *Bacillus thuringiensis* subsp. *kurstaki*.

Summary

Monsanto Company is submitting this Petition for Determination of Non-regulated Status to the Animal Plant Health Inspection Service (APHIS) regarding corn which expresses a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). This petition requests a determination from APHIS that insect protected corn line MON 80100, any progenies derived from crosses between MON 80100 and traditional corn varieties, and any progeny derived from crosses of MON 80100 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.

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 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 1992 through 1994 in the U.S. corn growing region under United States Department of Agriculture (USDA) permits or notifications as well as an Experimental Use Permit (524-EUP-82) obtained from the EPA in 1994. 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.

A series of insect protected corn lines derived from the plasmids identified within are currently under development and include MON 80100, MON 81000, and MON 81400, and MON 80900. However, the focus of this petition will be the insect protected corn line MON 80100. The determination that insect protected corn line MON 80100 and its progenies are no longer regulated articles and their subsequent commercialization will represent an efficacious and environmentally compatible addition to the existing options for corn insect pest management. The use of 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 ECB.
- Insect control without harming non-target species, including humans.
- A means for growers to significantly reduce the amount of chemical insecticides now applied to the crop thereby achieving ECB control in a more environmentally compatible manner than is currently available.

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

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

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

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

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

In conclusion, the consistent control afforded by insect protected corn line MON 80100 will enable growers to significantly reduce the amount of chemical insecticide now applied to their crop for control of ECB while maintaining yield potential. As a result, they will be able to utilize IPM practices that cannot presently be implemented because of the lack of options other than use of chemical insecticides to control this pest. An increase in the biological and cultural control of non-target corn pests and a more judicious use of chemical insecticides will result in a positive impact on the environment, which will ultimately be advantageous to the grower and the public as well.

Therefore, Monsanto Company requests a determination from APHIS that insect protected corn line MON 80100 and any progenies derived from crosses between MON 80100 and traditional corn varieties no longer be considered regulated articles under regulations in 7 CFR part 340.

Certification

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner which are unfavorable to the petition.

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Abbreviations Used in this Petition for the Determination of Non-Regulated Status of Insect Protected Corn Line MON 80100

2,4-D	(2,4-dichlorophenoxy)acetic acid
APHIS	Animal Plant Health Inspection Service
ATP	Adenosine triphosphate
bp, Kb	Base pairs, kilobase pairs
B.t.k.	*Bacillus thuringiensis* subsp. *kurstaki*
°C, °F	Degree Centigrade, degree Farenheight
CaMV	Cauliflower mosaic virus
CFR	Code of federal regulations
CP4 EPSPS	EPSPS from *Agrobacterium* sp. strain CP4
crylA(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
hsp70	Intron sequence from heat-shock protein 70
I-DNA	Integrated-DNA
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
NOS 3'	3' transcriptional termination sequence from nopaline synthase
NPTII	Neomycin phosphotransferase II
nptII	Gene for neomycin phosphotransferase II

ori-pUC	Bacterial origin of replication from the pUC plasmid
PCR	Polymerase chain reaction
ppm	Part per million
sp	Species
subsp.	Subspecies
T-DNA	Transferred-DNA
USDA	United States Department of Agriculture
w/w	Weight/weight

CONFIDENTIAL BUSINESS INFORMATION JUSTIFICATION

The information claimed as confidential within this petition falls into two categories, namely (1) the gene description (Figure IV.1) and (2) commercial development information (Appendices II and III). The gene description category includes information about the *cryIA(b)* gene. Commercial development information includes the names and locations of cooperators, collaborators, investigators, and contacts (referred within as "cooperators and collaborators").

LEGAL BACKGROUND

The Freedom of Information Act ("FOIA"), 5 U.S.C. § 552, specifically exempts from release "trade secrets and commercial or financial information obtained from a person and privileged or confidential" ("Exemption 4"). 5 U.S.C. § 552(b)(4). Exemption 4 applies where the disclosure of information would be likely to cause substantial harm to the competitive position of the owner, or where, in the case of voluntarily submitted information, the submitter would be less likely in the future to share data with the agency voluntarily. National Parks & Conservation Association v. Morton, 498 F.2d 765, 770 (D.C.Cir. 1974); Gulf & Western Industries, Inc. v. U.S., 615 F.2d 527, 530 (D.C.Cir. 1979).

A party seeking to demonstrate "substantial competitive harm" need not show actual competitive harm, but must only demonstrate the presence of competition and the likelihood of substantial competitive injury. Id. at 530; National Parks & Conservation Association v. Kleppe, 547 F.2d 673, 679 (D.C.Cir. 1976); Miami Herald Pub. Co. v. U.S. Small Business Administration, 670 F.2d 610, 614 (5th Cir. Unit B 1982).

For the purposes of FOIA, courts have defined the term "trade secret" to mean a "secret, commercially valuable plan, formula, process, or device that is used for the making, preparing, compounding, or processing of trade commodities and that can be said to be the end product of either innovation or substantial effort. Public Citizen Health Research Group v. FDA, 704 F.2d 1280, 1288 (D.C.Cir. 1983); Anderson v. Dept. of Health & Human Services, 907 F.2d 936, 943-44 (10th Cir. 1990).

Information on gene description and commercial development falls squarely within this definition, and is the type of information accorded trade secret protection by the courts under Exemption 4 of the Freedom of Information Act. It is well established that information on the formulation and chemistry of a product should be treated as confidential for FOIA purposes. See, e.g., Anderson v. Dept. of Health & Human Services, 907 F.2d 936 (10th Cir. 1990). This is exactly the type of information provided by each and every subcategory listed above in the gene description category. Where, as in the case of the Monsanto products subject to this FOIA request, the development time and costs of the product have been substantial and the information can only be obtained by competitors at considerable cost, disclosure is prohibited. Greenberg v. Food and Drug Administration, 803 F.2d at [213, 1216-1218 (D.C. Cir. 1986); Worthington Compressors, Inc. v. Costie, 622 F.2d 45, 51-52 (D.C.Cir. 1981). The existence of confidentiality agreements binding employees not to reveal the information is another factor considered by the courts. Greenberg v. FDA, 803 F.2d at 1216-1218.

The courts have also been very clear in finding commercial development information covered by Exemption 4 where the release of such information could allow competitors to procure a clear understanding of a company's business practices and allow a competitor to cause harm to a company's competitive standing. See, e.g., Braintree Electric Light Dept. v. Dept. of Energy, 494 F.Supp. 287, 289-291 (D.D.C. 1980). Information on distribution channels, market strategies, pricing structures, and patterns of competition fall squarely within the Exemption because such information enables a competitor to gain an accurate picture of a company's marketing activities and the competitive structure of the market. Timken v. U.S. Customs Service, 531 F.Supp. 194, 200 (D.D.C. 1981). Typically, information concerning marketing strategies, and the names of independent contractors participating in a company's studies have been accorded confidential treatment. See, e.g., Teich v. Food & Drug Administration, 751 F.Supp. 243, 253 (D.D.C. 1990).

In a case recently decided by the U.S. Court of Appeals for the District of Columbia Circuit, Critical Mass Energy Project v. NRC, No. 90-5120, August 21, 1992, the court determined that information given to the government voluntarily will be treated as confidential under Exemption 4 if such information is of the kind that the provider would not customarily make available to the public. To the extent any references and other information in the Monsanto applications were submitted voluntarily, such information is accorded protection from disclosure.

GENE DESCRIPTION

The essence of the commercial value of the Monsanto biotechnology products is the particular genetic information that confers the desired properties on the plant product, as well as the technical know-how inherent in this information. Monsanto is at the leading edge in the development of biotechnology products in a rapidly growing and highly competitive industry. This expertise has been gained through many man years of effort, and the expenditure of tens of millions of dollars on biotechnology research.

Monsanto has been working on the development of insect protection since the early 1980's and has expended several million dollars in research and testing costs. Monsanto can document the development and testing costs by means of monthly summaries of the man hours devoted to these projects, budgetary documents, field test agreements, and project documents for the Chesterfield facility.

The uniqueness of Monsanto's product lies in the particular uniqueness of genetic components in the vectors transferred to these plants. Each genetic entity in these vectors has three pieces of information: a promotor region, the gene for the expression of the trait, and a stop signal. Although the information on the *cryIA(b)* gene may be in the public domain, the particular genetic elements assembled by Monsanto are unique and represent years of effort and millions of dollars of expense.

To achieve the product which is the subject of this Confidential Business Information Justification, Monsanto has developed and tested many different plant strains using different combinations of genetic components. The plant products developed by Monsanto represent the best fit of the components, and the best mode of gene expression of the desired insect protection trait. The specific combination of genetic information on the vectors transferred to the Monsanto products has been kept strictly confidential. Monsanto employees and contractors under contract to Monsanto are contractually obligated to keep this information confidential.

There are many competitors of Monsanto, both national and international, who have the expertise not only to replicate Monsanto's products, but also to use Monsanto's technology to develop other products which would be competitive with Monsanto, thereby saving millions of dollars and years of development effort.

Monsanto's competitors cannot presently duplicate Monsanto's commercially valuable products from information in the public domain without going through the same painstaking trial and error development and testing of many different combinations of genetic information. It is important to emphasize that although there may be information about Monsanto products available in patent applications, this information is voluminous and general in nature, and does not identify the specific combinations of genetic information which Monsanto has found to be most effective. A competitor cannot determine from the patent applications which particular combination of genes and transgenic products will prove to be commercially valuable.

Access to gene description information for Monsanto's products would allow competitors to create essentially "copy-cat" products (avoiding any technical patent infringement) that would result in a market share loss for Monsanto of millions of dollars. By performing simple copy work, these competitors would avoid the millions of dollars and many years of research and development effort expended by Monsanto to develop its commercial products.

The release of gene description information would also provide competitors with commercially valuable knowledge about the particular products that Monsanto is planning to commercialize and the likely time frame for commercialization. This information would be extremely helpful to these companies in developing their own marketing strategies and development plans in a highly competitive market.

NAMES AND INFORMATION ABOUT GENES, PROMOTERS AND EXPRESSED TRAITS

The release of information about the gene in the vector identified will directly provide competitors with the knowledge of the precise genetic sequence that Monsanto has found to be most desirable. If this information is disclosed, the competitors will have access to the structure of the Monsanto products, with the consequences outlined above. Patents for the products at issue in this matter are pending, but have not been issued.

Information on the expressed trait of the genes is tantamount to providing the name of the genes, and will allow Monsanto's competitors to readily identify the particular genes that have been transferred to the Monsanto products. The release of any information relating to changes made to an original gene to facilitate fusion with another gene would explicitly reveal Monsanto's trade secret technology for developing gene combinations.

COMMERCIAL DEVELOPMENT INFORMATION

The disclosure of information about the names of cooperators, collaborators, investigators, research farm on-site personnel or contacts ("cooperators and collaborators") and the location and characteristics of the field experiments will provide Monsanto's competitors with invaluable information about Monsanto's marketing strategy, and could cause severe harm to Monsanto's competitive standing in the industry.

COOPERATORS AND COLLABORATORS AND INFORMATION LEADING TO THEIR IDENTITY

In particular, information about the choice of cooperators and collaborators provides the competition with knowledge about the individuals and organizations that Monsanto has found, through its experience and investigation, to be most expert. Without doubt, the competitors would seek to use the services of the entities found most expert by Monsanto, thereby driving up the price of services for future Monsanto contracts with these entities.

Moreover, since there is a limitation on the expertise and facilities necessary to test and produce the Monsanto products, the competitors could use information on the cooperators and collaborators to block or limit access of these sources of expertise to Monsanto. This could be accomplished either by the competitors acquiring exclusive licenses with these individuals and organizations, or by their entering into contracts that would utilize a substantial amount of the time and facilities of such entities.

Maintaining the good will of the cooperators and collaborators is also a very important consideration for the success of Monsanto's marketing strategy. The release of information identifying or leading to the identity of these entities could cost Monsanto considerable good will. Monsanto has entered into an agreement with a number of cooperators that it would keep the names of these cooperators confidential. The release of information about these cooperators would likely jeopardize Monsanto's relationship with these cooperators, and might prevent the cooperators from working with Monsanto in the future. Good will is established through working relationships with cooperators, irrespective of whether there is a written agreement between Monsanto and such cooperators. If Monsanto has given its word that it would maintain confidentiality, a loss of good will follow from the breach of this agreement.

If Monsanto were forced to use alternative cooperators and collaborators, it would not only be losing access to the best technical performance, but would lose the value of the expertise built-up by these entities from prior work with Monsanto products. The delay in bringing products to market could be considerable for Monsanto, and the cost could be in the millions.

To the extent Monsanto has not finalized its contract with a collaborator or cooperator, the release of the name of this individual or entity could dramatically increase the contract price, or make contractual arrangements very difficult to achieve. This would result from the competitive pressure on the collaborators or cooperators by other companies seeking to capitalize on the Monsanto technology. A cooperator or collaborator is also more likely to seek an excessive price for his technology if he is aware that his product is close to commercial production.

In addition, the disclosure of information about cooperators and collaborators would provide strong insights into Monsanto's marketing strategy by revealing where Monsanto is planning to introduce the products, and the schedule for such introduction.

PETITION FOR DETERMINATION OF NON-REGULATED STATUS OF INSECT PROTECTED CORN LINE MON 80100

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Part I. Rationale for Development of Insect Protected Corn

A. Need for Insect Protected Corn

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

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

Monsanto has developed genetically modified corn plants that control ECB. This insect protected corn offers a new mechanism to produce and deliver a highly effective insecticide to target pests (e.g. production by cells of the crop plant rather than industrially and application by spray equipment). The technology couples the environmental advantages of host plant resistance with the efficacy of CryIA(b), an effective biological

insecticide. Insect protected corn expresses the CryIA(b) protein which is selective against certain lepidopteran insects that must feed upon the plants to be controlled. Therefore, this technology offers selective activity without disrupting pest suppression by natural enemies, such as parasites and predators.

B. Benefits of Insect Protected Corn

Agronomic Benefits of Corn Genetically Modified to Resist European Corn Borer and Other Lepidopteran Pests

Kevin L. Steffey, Professor
Office of Agricultural Entomology
University of Illinois
Champaign-Urbana, IL

Executive Summary

Insect protected corn will provide excellent control of an insect (European corn borer) that causes significant decreases in corn yields every year in North America. Results from field experiments with Monsanto's genetically modified corn expressing delta-endotoxin proteins of *Bacillus thuringiensis* var. *kurstaki* (insect protected corn) revealed a high level of efficacy against European corn borers, *Ostrinia nubilalis* (Hübner), in 1994. The results from field trials in Kansas indicated that Monsanto's transgenic corn also is efficacious against southwestern corn borers, *Diatraea grandiosella* Dyar. Southwestern corn borers occur in areas of the western Corn Belt where this pest causes yield reductions. Control of other lepidopteran pests with insect protected corn may also be possible, and is currently being investigated for armyworms, corn earworms, fall armyworms, and stalk borers in corn.

Currently, corn growers in the eastern Corn Belt treat relatively few acres annually with insecticides to control European corn borers. Corn growers in Colorado, Kansas, and Nebraska treat comparatively more acres to control corn borers. However, yield losses attributable to corn borer damage are appreciable throughout its range. One study in Illinois (Briggs & Guse 1986) revealed that approximately 10 percent of the corn acres in that state experience a 9- to 15-percent yield loss annually, attributable solely to the damage caused by the second generation of corn borers. [At least two generations of this pest occur annually throughout most of the Corn Belt.] Results from several studies suggest that corn borers cause an estimated 5 to 7.5 percent yield loss annually (first and second generations combined) (Gray

and Steffey 1995; Bergman et al. 1985a-f; Bode and Calvin 1990). These data induce entomologists throughout the United States to consider the European corn borer to be the most under-scouted and under-treated insect that attacks corn. Because European corn borers cause primarily physiological reductions in yield, corn growers are not aware of the significance of their feeding injury during years when infestations are moderate. In addition, efficacy of insecticides applied for control of corn borers is often less than acceptable, particularly for the second generation. Both timing of insecticide applications and placement of the insecticide where corn borer larvae are feeding are difficult. Corn growers frequently are dissatisfied with the level of control of corn borers provided by both chemical and microbial insecticides.

The only other management tactic currently utilized for management of European corn borers is planting of resistant or tolerant corn hybrids. Entomologists and corn breeders have attempted for many years to develop hybrids resistant to European corn borers. However, although some hybrids are resistant to first-generation corn borers, none are resistant to second-generation borers. Some hybrids also have the ability to tolerate an infestation of corn borers. Nevertheless, planting of corn hybrids specifically because they are resistant to European corn borers is not widespread, and tolerant hybrids often do not yield as well when infestations of corn borers are heavy.

Insect protected corn promises to be a profound breakthrough in corn insect management. Corn growers who plant insect protected corn will experience yield protection during years when infestations of European corn borers are moderate to large. The potential for substantial reduction or virtual elimination of insecticide use for corn borer control is real. Additionally, the selective activity of the *Btk* endotoxins will not disrupt populations of either beneficial insects or nontarget animals (e.g., birds, fish). Applications of conventional chemical insecticides often affect nontarget species.

The development of insect protected corn may become a foundation for corn insect management throughout the United States. Reduced insecticide use and improved yields are the likely outcomes of implementation of this technology. If growing insect protected corn effectively eliminates all insecticide applications for European corn borers, corn growers would save a conservative $50 million annually. [This figure was derived from an estimate of 5 percent of the acres of corn treated with insecticides for corn borer control and $15 per acre control costs (insecticide + application costs).] The yield protection benefits gained from controlling corn borer infestations are

between 1 and 1.5 billion dollars. [This figure was derived from annual estimates of 70 million acres of corn, an average yield of 120 bushels per acre, an average corn price of $2.35 per bushel, and an estimated 5 to 7.5 percent yield loss attributed to corn borer damage.]

The development of insect protected corn will have a major impact on corn pest management. The reduction in the use of aerially applied insecticides will preserve many beneficial insects, and the integration of insect protected corn with other forms of resistance or tolerance will provide solid footing for the development of nonchemical technologies for other major insect pests.

A complete copy of the agronomic benefits document is located in Appendix I.

C. Regulatory Approvals

Before commercializing the insect protected corn line MON 80100, Monsanto will seek the following regulatory approvals:

1. This determination from USDA/APHIS that insect protected corn line MON 80100, and all progenies from crosses between insect protected corn line MON 80100 and other corn varieties, is 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). This petition has been submitted.

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

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

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

D. References

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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 described by Doebley and Iltis (1980) will be used herein.

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 were provided by Coe et al. (1988) on corn genetics, by Carlson (1988) on corn cytogenetics, by Phillips et al. (1988) on cell tissue culture, and by Walbot and Messing (1988) on molecular genetics. 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. Wilkes (1972) reported 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. As stated by Wilkes (1972), this frequency of hybrids represents a significant gene exchange between a wild weedy plant (i.e., teosinte) and a cultivated relative (i.e., corn). 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 Galinat (1988) has mapped more than 50 homologous loci on the chromosomes of corn and *Tripsacum*. 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)
australe (2n = 36)
bravum (2n = 36, 72)
cundinamarce (2n = 36)
dactyloides (2n = 72)
floridanum (2n = 36)
intermedium (2n = 72)
manisuroides (2n = 72)
latifolium (2n = 36)
percuvianum (2n = 72, 90, 108)
zopilotense (2n - 36, 72)
jalapense (2n = 72)
lanceolatum (2n = 72)
laxum (2n = 36?)
maizar (2n = 36, 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

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Part III. Description of the Transformation System and Plasmids Utilized

Introduction

Insect protected corn line MON 80100 for which this determination is requested, contains the following four genes inserted via genetic engineering techniques:

- The *cryIA(b)* gene encodes for an insecticidal protein, CryIA(b), derived from the common soil microbe *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*).

- The CP4 EPSPS gene encodes the selectable marker enzyme 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and was used as a plant selectable marker in corn plant transformation, allowing the selection of genetically modified cells on media containing glyphosate. It served no other purpose and has no pesticidal properties.

- The *gox* gene, encoding the selectable marker enzyme glyphosate oxidoreductase (GOX), was also used as a plant selectable marker. Although the *gox* gene sequence was present, the GOX protein was not detected by ELISA or western analyses.

- The *nptII* gene encodes the bacterial selectable marker enzyme neomycin phosphotransferase II (NPTII) and allowed for the selection of bacteria containing the PV-ZMBK07 or PV-ZMGT10 plasmids in media containing aminoglycoside antibiotics (i.e., kanamycin or neomycin). The *nptII* gene is under the control of a bacterial promoter and as expected, the encoded protein was not detected in the insect protected corn line MON 80100.

The described *cryIA(b)* gene and marker genes were introduced into corn by a particle acceleration method. This system and the properties of the non-transformed corn cultivar are described below.

A. Properties of the Non-transformed Cultivar

The genetic material that is the recipient of the added genes is a derivative of the A188 and B73 inbred lines of corn. These are publicly-available inbred lines developed by the University of Minnesota and Iowa State University, respectively. Designated "Hi-II", the recipient material is approximately 50:50 of the two lines (Armstrong *et al*, 1991). The material was developed to have a higher regeneration potential (from the combinations of genes from A188 and B73)) along with acceptable commercial performance in hybrids (from B73).

B. Particle Acceleration Transformation System

DNA was introduced into the plant tissue by the particle acceleration method described by 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 are assayed for the presence of the expressed CryIA(b) protein product.

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

The corn genotype Hi-II was transformed with two plasmid vectors, PV-ZMBK07 and PV-ZMGT10, using the particle acceleration method identified above. The PV-ZMBK07 plasmid contains the *cryIA(b)* gene and PV-ZMGT10 contains the CP4 EPSPS and *gox* genes. Both plasmids contain the *nptII* gene under the control of a bacterial promoter and an origin of replication from a pUC plasmid, required for selection and replication in bacteria, respectively. The plasmid vector PV-ZMBK07 is shown in Figure III.1 and PV-ZMGT10 is shown in Figure III.2. A description of the DNA elements in PV-ZMBK07 and PV-ZMGT10 are given in Tables III.1 and III.2, respectively.

1. **Plant Expression Vector - PV-ZMBK07**

The plasmid vector PV-ZMBK07 (Figure III.1) contains the *cryIA(b)* gene under the control of the enhanced 35S promoter (E35S) (Kay *et al*, 1985 and Odell *et al*, 1985), which is approximately 0.6 Kb in size. Located between the enhanced 35S promoter and the *cryIA(b)* gene is the 0.8 Kb intron from the *hsp70* gene (heat-shock protein), present to increase the levels of gene transcription (Rochester *et al*, 1986). The *cryIA(b)* gene is joined to the 0.24 Kb nopaline synthase 3' nontranslated sequence, NOS 3', (Rogers *et al*, 1985) which provides the mRNA polyadenylation signals.

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 the nature identical CryIA(b) *B.t.k.* HD-1 protein product (Fischhoff *et al*, 1987). The deduced amino acid sequence for the *cryIA(b)* gene is given in Figure IV.3.

The *alpha* region of the *lacZ* gene for beta-galactosidase, present under a bacterial controlled promoter, is present in PV-ZMBK07. 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). Most of the approximately 200 bp region of the alpha region of the gene for beta-galactosidase was not incorporated into corn line MON 80100. The *lacZ-alpha* region is followed by the 0.7 Kb origin of replication for the pUC plasmids (*ori-pUC*) and which allows for the replication of plasmids in *E. coli* (Vieira and Messing, 1987).

Following the *ori-pUC* region is the gene for the enzyme neomycin phosphotransferase type II (NPTII). This enzyme confers resistance to aminoglycoside antibiotics (*i.e.*, kanamycin and neomycin) and was used for 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 NPTII protein was not detected in corn line MON 80100 (Ream *et al*, 1995). The deduced amino acid sequence for NPTII is given in Figure IV.6.

2. Plant Expression Vector - PV-ZMGT10

The PV-ZMGT10 plasmid (Figure III.2) contains the *gox* and CP4 EPSPS genes joined to chloroplast transit peptides CTP1 and CTP2, respectively. Both coding regions are under the control of the enhanced 35S promoter, *hsp70* intron and NOS 3' terminator sequences. The PV-ZMGT10 vector contains the same *lacZ-alpha*, *ori-pUC* and *nptII* regions as described above for PV-ZMBK07. The CP4 EPSPS and *gox* genes enable the selection of cells in tissue culture that contain the *cryIA(b)* gene.

A CP4 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) and 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). Upon glyphosate treatment, 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.

The CP4 EPSPS gene in PV-ZMGT10 contains a chloroplast transit peptide, CTP2, isolated from *Arabidopsis thaliana* EPSPS (Klee and Rogers, 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). The CP4 EPSPS gene with its CTP2 is approximately 1.7 Kb in size. The CP4 EPSPS gene cassette (promoter through 3' termination sequence) is joined to the *gox* cassette. The amino acid sequence of CP4 EPSPS with its CTP is given in Figure IV.5.

The *gox* gene that encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX) was cloned from *Achromobacter* sp. strain LBAA (Hallas *et al*, 1988; Barry *et al*, 1992; Barry *et al*, 1994). The GOX protein is targeted to the plastids with a chloroplast transit peptide sequence, CTP1. The CTP1 was derived from the small subunit gene of ribulose-1,5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* (Timko *et al*, 1988). The enzyme GOX degrades glyphosate by converting glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate. The GOX protein was not detected by ELISA or western blot analysis in the seeds or leaves of the corn line, MON 80100. The amino acid sequence of GOX with its CTP is given in Figure IV.4.

Table III.1 Summary of DNA components in the plasmid PV-ZMBKO7.

Genetic Element	Size, Kb	Function
P-E35S	0.62	The cauliflower mosaic virus (CaMV) promoter (Odell *et al*, 1985) with the duplicated enhancer region (Kay *et al*., 1985).
hsp 70 intron	0.80	Intron from the *hsp70* gene (heat-shock protein) present to increase the levels of gene transcription (Rochester *et al*, 1986).
cryIA(b)	3.5	The gene which confers tolerance to ECB. The gene encodes the nature identical CryIA(b) *B.t.k.* HD-1 protein product (Fischoff et al, 1987).
NOS 3'	0.24	A 3' nontranslated region of the nopaline synthase gene which functions to terminate transcription and direct polyadenylation (Depicker *et al*, 1982; Bevan, 1984).
lacZ-alpha	0.24	The gene for the alpha region of beta-galactosidase under its bacterial controlled promoter used for plasmid construction in bacteria (Vieira and Messing, 1987).
ori-pUC	0.67	The origin of replication for the pUC plasmids that allows for plasmid replication in *E. coli* (Vieira and Messing, 1987).
npt II	0.79	The gene for the enzyme neomycin phosphotransferase, type II. This enzyme confers resistance to aminoglycoside antibiotics and thereby allows for plasmid selection in bacteria (Beck *et al*, 1982).

Table III.2 Summary of DNA components in the plasmid PV-ZMGT10.

Genetic Element	Size, Kb	Function
P-E35S	0.62	The cauliflower mosaic virus (CaMV) promoter (Odell *et al*, 1985) with the duplicated enhancer region (Kay *et al*, 1985).
hsp 70 intron	0.80	Intron from the *hsp70* gene (heat-shock protein) present to increase the levels of gene transcription (Rochester *et al*, 1986).
CTP2	0.31	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS, 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) which allows for the selection of transformed cells on glyphosate.
NOS 3'	0.24	A 3' nontranslated region of the nopaline synthase gene which functions to terminate transcription and direct polyadenylation (Depicker *et al*, 1982; Bevan *et al*, 1984).
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 that encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX), isolated from *Achromobacter* sp. strain LBAA (Hallas *et al*, 1988; Barry *et al*, 1992) Barry *et al*, 1994), which allows for the selection of transformed cells on glyphosate.
lacZ-alpha	0.24	The gene for the alpha region of beta-galactosidase under its bacterial controlled promoter used for plasmid construction in bacteria (Vieira and Messing, 1987).
ori-pUC	0.67	The origin of replication for the pUC plasmids that allows for plasmid replication in *E. coli* (Vieira and Messing, 1987).
npt II	0.79	The gene for the enzyme neomycin phosphotransferase, type II. This enzyme confers resistance to aminoglycoside antibiotics and thereby allows for plasmid selection in bacteria (Beck *et al*, 1982).

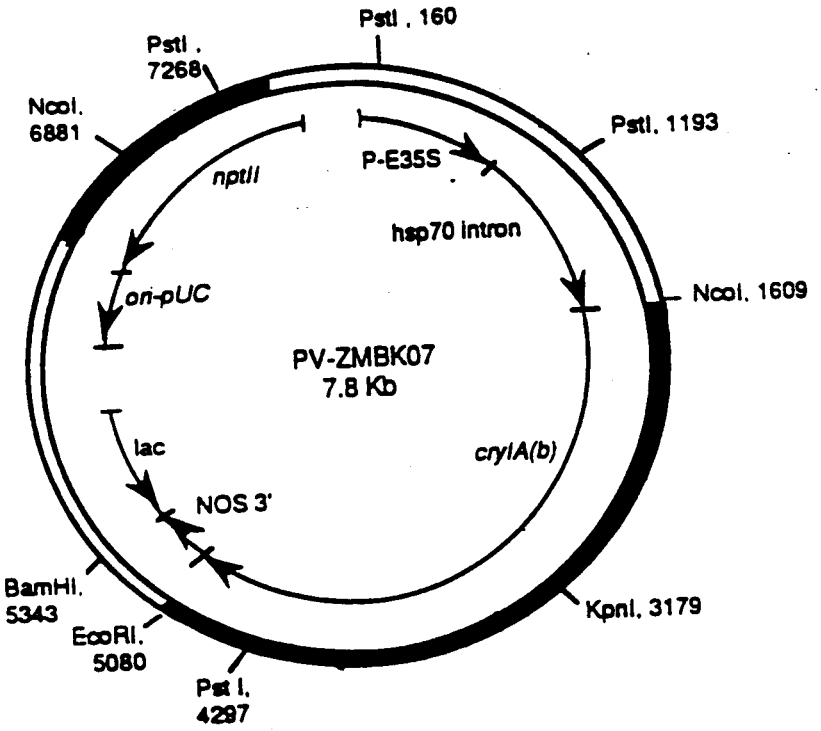

Figure III.1. Plasmid map of PV-ZMBK07. Restriction sites, and their locations in bp, utilized during Southern analyses are shown. The blackened regions denote the positions of homology for probes used during Southern analyses.

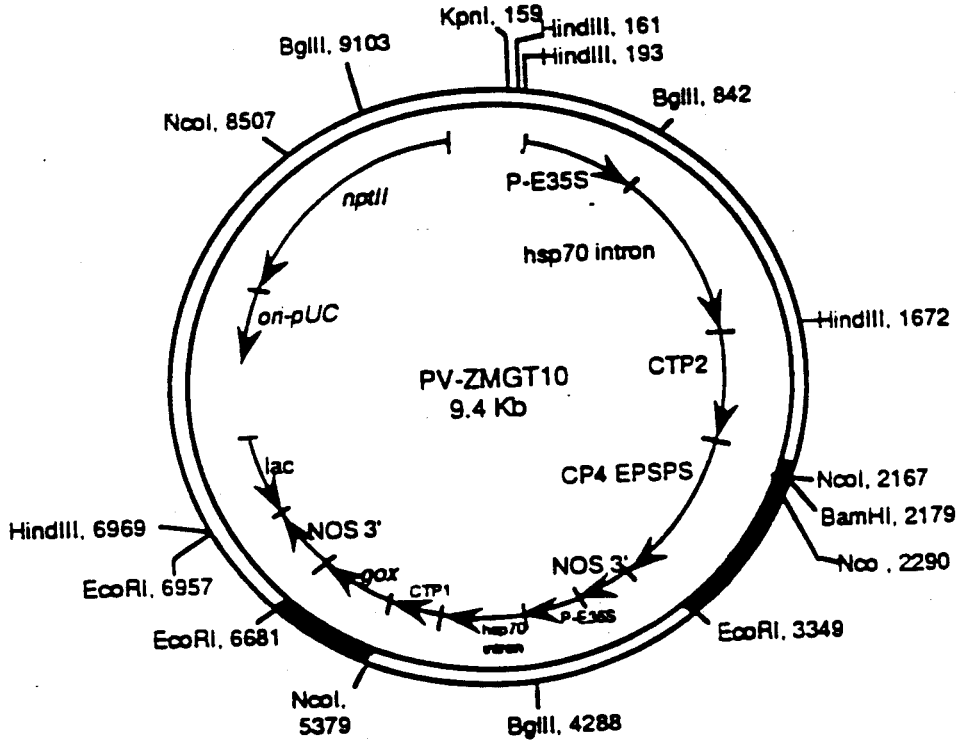

Figure III.2. Plasmid map of PV-ZMGT10. Restriction sites, and their locations in bp, utilized during Southern analyses are shown. The blackened regions denote the positions of homology for probes used during Southern analyses.

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Part IV. The Donor Genes to be Considered for Non-regulated Status and Molecular Biology of Insect Protected Corn Line MON 80100

Introduction

As described in Part III, the insect protected corn line MON 80100 was generated using a particle acceleration transformation system with plasmids PV-ZMBK07 and PV-ZMGT10. The *cryIA(b)* gene was inserted to confer resistance to certain lepidopteran insects while the CP4 EPSPS and *gox* genes produce proteins which confer tolerance to glyphosate, a selective agent used to identify plant cells expressing the *cryIA(b)* gene. In addition to these three genes, a *nptII* gene which produces the enzyme neomycin phosphotransferase II (NPTII) was introduced under the control of its own bacterial promoter, to enable selection in bacterial systems.

DNA analysis was performed to characterize the integrated DNAs (I-DNAs) present in the corn line MON 80100, that express the full length CryIA(b) protein. The I-DNAs were evaluated using Southern (Southern, 1975) blot analyses for the number of sites into which the plasmid DNA integrated into the corn genome, and for the number of copies and integrity of the genes contained within the I-DNAs.

A. The Donor Genes to be Considered for Non-regulated Status

1. The *cryIA(b)* Gene

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

The nucleotide sequence of the gene was modified to enhance expression in corn and is shown in Figure IV.1. However, the amino acid sequence of the encoded protein was maintained to be identical to the the naturally produced protein (Figure IV.3) (Perlak *et al*, 1991).

2. The CP4 EPSPS Marker Gene

The CP4 EPSPS gene was used as a plant selectable marker to identify the rare corn cells that originally received the introduced *cryIA(b)* gene responsible for insect protection in the initial, laboratory stages of plant cell selection following transformation. The mode of action of glyphosate is the inhibition of the enzyme 5-enolpyruvyl-shikimate-3-phosphate

synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinruken *et al*, 1980). Corn plants tolerant to glyphosate at the laboratory level were produced by stably inserting the CP4 EPSPS and/or *gox* genes into the chromosome of corn.

The CP4 EPSPS gene from *Agrobacterium* sp. strain CP4, as contained in plasmid PV-ZMGT10, is shown in Figure IV.2. The gene for CP4 EPSPS 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). Upon glyphosate treatment, the corn cells expressing the CP4 EPSPS are tolerant because the CP4 EPSPS continues to meet the plant's need for aromatic compounds in the presence of glyphosate. 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 plant produced EPSPSs are present in the chloroplast. Therefore, the chloroplast transit peptide coding sequence, CTP2, from *Arabidopsis thaliana* EPSPS (Klee *et al*, 1987) was fused to the N-terminus of the CP4 EPSPS protein to deliver the CP4 EPSPS to the chloroplasts, the site of EPSPS activity and glyphosate action (Kishore and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (della Cioppa *et al*, 1986). The deduced amino acid sequence of the CP4 EPSPS protein including CTP2 is given in Figure IV.5.

3. The *gox* Marker Gene

The *gox* gene, cloned from *Achromobacter* sp. strain LBAA was also inserted as a selectable marker in plants. The *gox* gene sequence, as contained in plasmid PV-ZMGT10, is given in Figure IV.2. The GOX gene encodes a 46.1 kD protein and was isolated from *Achromobacter* sp. strain LBAA (Barry *et al*, 1992). The GOX protein is targeted to the plastids with a chloroplast transit peptide sequence, CTP1 derived from the SSU1A gene from *Arabidopsis thaliana* (Timko *et al*, 1988). GOX degrades glyphosate by converting glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate and has no pesticidal effect. Although the *gox* gene sequence is present incuding the CTP, the GOX protein has not been detected by ELISA or western analysis in the seeds or leaves of this lead corn line, MON 80100. The deduced amino acid sequence of GOX containing CTP1 is shown in Figure IV.4.

4. The *nptII* Gene

The bacterial selectable marker gene, *nptII* , isolated from the prokaryotic transposon, Tn5 (Beck *et al*, 1982), encodes for the enzyme neomycin phosphotransferase II which confers resistance to the aminoglycoside antibiotics (i.e., kanamycin or neomycin) used for selection of plasmids in *E. coli*. The promoter for this gene is only active in bacterial hosts. As expected, no NPTII protein was detected in the insect protected corn line MON 80100 using western blot analysis.

The *nptII* gene sequence in plasmids PV-ZMBK07 and PV-ZMGT10 is shown in Figures IV.1 and IV.2, respectively. The deduced amino acid sequence is given in Figure IV.6.

B. Genetic Analysis of Insect Protected Corn Line MON 80100

1. Western Results

During the process of particle acceleration, the plasmid DNA can become broken resulting in the integration of partial genes into the genomic DNA. Therefore, western analyses of the protein products for the three integrated plant-expressed genes, *cryIA(b)*, CP4 EPSPS and *gox*, were conducted using tissue derived from the corn line MON 80100. Western blot analysis enabled the evaluation of the presence and sizes of the expressed proteins using antibodies specific to the protein under examination. Comparisons to protein standards (isolated from *E. coli)* and tissue extracts from other insect protected corn lines containing the same genes were made in order to evaluate if the produced proteins were of the expected size and if any unexpected protein products were made.

The expected size 131 kD CryIA(b) and 47.6 kD CP4 EPSPS proteins were observed in tissue extracts from line MON 80100 (compared to the standard isolated from *E. coli)* with no other detectable protein products (compared to other lines containing the same genes). The GOX protein was not detected (down to a sensitivity of 0.0033%) in line MON 80100.

a. CryIA(b) Protein

Leaf extracts from the corn line MON 80100 and two other insect protected corn lines, MON 80200 and MON 80400, were compared to a *B.t.k.* protein standard, *B.t.k.* HD-73 (Figure IV.7, lanes 1, 3, 4 and 5). The full length *B.t.k.* HD-73 standard is approximately 134 kD in size and the full length CryIA(b) protein standard is approximately 131 kD in size. The *B.t.k.* HD-73 standard was used due to its availability and is an appropriate standard for these analyses (Lee *et al*, 1995a).

As is commonly observed in the western analysis of *B.t.* proteins (Lee et al, 1995a), multiple protein products were observed for all three insect protected corn lines (Figure IV.7 lanes 3, 4 and 5). Line MON 80200 clearly showed the full length CryIA(b) protein product at approximately 131 kD (Figure IV.7, lane 3). The full length CryIA(b) protein was difficult to distinguish for lines MON 80400 and MON 80100 (Figure IV.7 lanes 4 and 5, respectively), due to low levels of the full length protein in these tissues. However, upon subjecting the protein extracts to trypsin, all three lines showed the common CryIA(b) trypsin-resistant core protein product at approximately 63 kD (Figure IV.7, lanes 7, 8, 9 and 10), as previously reported (Lee *et al*, 1995b). The antibody is known to have increased sensitivity with the tryptic-core protein compared to the full length protein (Lee *et al*, 1995a). In all three lines evaluated, no detectable qualitative differences in the non-trypsinized or trypsinized treated extracts were observed.

Therefore, it was concluded that the trypsin-resistant core protein produced in line MON 80100 and two other insect protected corn lines were the same size as that observed for the CryIA(b) protein standard and that there were no unexpected immuno-reactive protein products. Additionally, the CryIA(b) protein produced in line MON 80100 was indistinguishable from the CryIA(b) protein produced in two other insect protected corn lines produced with the same *cryIA(b)* gene.

b. CP4 EPSPS Protein

Extracts from whole plant tissue of the corn line MON 80100 and another insect protected corn line 523-06-01 (a line previously shown to express CP4 EPSPS, Lee *et al*, 1995c) were compared to a CP4 EPSPS protein standard (Figure IV.8, lanes 1, 4 and 5). Protein extracts from whole plant (WP) tissues of insect protected corn lines MON 80100 and line 523-06-01 were found to produce the expected 47.6 kD protein product when compared to the purified CP4 EPSPS protein standard. The extracts from the control line, Hi-II/FRB73 contained a protein just above the 47.6 kD band observed in the *E. coli* produced standard. Since this appeared in the control and insect protected corn line extracts, it was concluded to be background (*i.e.*, not a CP4 EPSPS protein).

In both the whole plant and seed extracts of line MON 80100, only the CP4 EPSPS protein product of the correct size was observed (Figure IV.8, lanes 5 and 8, respectively). Therefore, it was concluded that the CP4 EPSPS protein produced in line MON 80100 was of the expected size (compared to the *E. coli* produced standard) and indistinguishable from the CP4 EPSPS protein produced in corn line 523-06-01, another insect protected corn line produced with the same CP4 EPSPS gene.

c. GOX Protein

Leaf extracts from the corn line MON 80100 and another insect protected corn line MON 80200 (a line previously shown to express the GOX protein) were compared to a GOX protein standard. As shown in Figure IV.9, lane 5, the GOX protein standard was detectable at a level of 1 ng spiked into 30 µg of total protein extract (0.0033%). Tissue extract from line MON 80200 showed the presence of the GOX protein when 3 µg of total protein was analyzed (Figure IV.9, lane 10). When 30 µg of total protein extracted from line MON 80100 was analyzed, no GOX protein was detected (Figure IV.9, lane 9). We conclude that the *gox* gene expresses no detectable GOX protein in MON 80100. The absence of any detectable GOX protein in the seed, leaf or whole plant extracts of MON 80100 was confirmed by ELISA (Ream, *et al*, 1995).

In summary, the CryIA(b) and CP4 EPSPS proteins produced in insect protected corn line MON 80100 are of the correct size and are indistinguishable from other lines produced with the same respective genes. The GOX protein was not detected (down to a sensitivity of 0.0033%) in leaf tissue from line MON 80100.

2. Southern Results

Two plasmid vectors were utilized during the particle acceleration process to produce the corn line MON 80100. Plasmid PV-ZMBK07 contains the *cryIA(b)* gene and plasmid PV-ZMGT10 contains the CP4 EPSPS and *gox* genes. The maps of the two plasmid vectors are presented in Figures III.1 and III.2 along with the locations of the restriction sites utilized during Southern analyses. A further description of the sizes and sources of the genes within the two vectors is given in Part III.C. The DNA sequence of plasmids PV-ZMBK07 and PV-ZMGT10 are given in Figures IV.1 and IV.2, respectively.

The DNA from the control line MON 80080 and line MON 80100 were digested with a variety of different restriction enzymes and subjected to Southern blot hybridization analyses to characterize the DNA that was transferred from the coated particles during particle acceleration into the corn genome. Specifically, the insert number (number of integration sites within the corn genome), copy number (number of each gene within the integrated DNAs) and the insert integrity were examined.

In addition to Southern blot analyses, a custom genomic library was constructed using genomic DNA from line MON 80100. Clones from the library spanning the I-DNAs were isolated and used for sequence analysis of the segments of the I-DNA in which portions of the two

different plasmid fragments were joined (junction segments). An internal junction is defined as a site where one plasmid broke and joined with another plasmid (either a segment from the same plasmid or the other plasmid) and an external junction is defined as the end of the I-DNA (the junction of the I-DNA and the genomic DNA).

From Southern analyses described below, two I-DNAs were observed within the genome of line MON 80100. Figures IV.10 and IV.11 are schematic representations of the genetic composition of the two I-DNAs. Insert number 1, approximately 5.5 Kb in size, contains a partial *cryIA(b)* gene and a full length *gox* gene (Figure IV.10). Insert number 2, approximately 19 Kb in size, contains a full length *cryIA(b)* gene, two partial and one full length CP4 EPSPS genes, one partial *gox* gene and a partial and full length *nptII* genes. A partial gene is defined as a gene that is less than full length. The internal and external junction fragments were sequenced to verify the sequence of junction sites within the two I-DNAs. The information provided below supports the elucidated structure of the I-DNAs presented in Figures IV.10 and IV.11.

a. Insert Number (number of I-DNAs)

In order to determine the number of insertion events, the DNA from line MON 80100 and the parental control line MON 80080 were digested with the restriction enzyme *Nde*I, which does not cleave within either of the plasmids (PV-ZMBK07 or PV-ZMGT10) used to produce line MON 80100. The results of hybridizing the blot with a mixture of radiolabelled plasmid probes (PV-ZMBK07 and PV-ZMGT10) or with the individual *cryIA(b)*, *gox* and CP4 EPSPS gene specific probes are shown in Figure IV.12. The results presented in Figure IV.12 represent a composite of several blots that were probed separately and then combined. The hybridization done with the plasmid probes indicated that the plasmid DNA integrated at two separate sites within the corn genomic DNA to produce line MON 80100; this was concluded by observing the presence of two hybridizing DNA fragments of approximate sizes 26 and 20 Kb (Figure IV.12, lane 2). Two other bands were observed in Figure IV.2, lane 2 but were also observed in the control DNA (Figure IV.12, lane 1) and therefore concluded to be background hybridization. Background bands are defined as DNA fragments within the control and test samples that hybridize to the probe and therefore are not considered as resulting from the integrated DNA.

The 26 Kb *Nde*I fragment is referred to as insert number 1 and the 20 Kb *Nde*I fragment is referred to as insert number 2. The *cryIA(b)* gene probe hybridized to both of the fragments (Figure IV.12, lane 4)

although the intensity of the hybridization was less with insert number 1 than for insert number 2, suggesting that less of the *cryIA(b)* gene integrated within the 26 Kb fragment (insert number 1) than the 20 Kb fragment (insert number 2). The *gox* probe also hybridized to both fragments (Figure IV.12, lane 6) but the intensity of the hybridization was less on insert number 2 than on insert number 1 suggesting that less of the *gox* gene integrated within the 20 Kb fragment than in the 26 Kb fragment. The CP4 EPSPS gene probe only hybridized to the 20 Kb fragment (Figure IV.12, lane 8) establishing that the CP4 EPSPS gene was only present in insert number 2.

Therefore, this data established that two DNA fragments integrated into the corn genome. These were defined as insert number 1 and insert number 2. Based on the relative hybridization band intensities, it was concluded that insert number 1 contained a less than full length *cryIA(b)* gene and a *gox* gene and insert number 2 contained a *cryIA(b)* gene (greater in size than on insert number 1), a CP4 EPSPS gene and a less than full length *gox* gene.

In order to elucidate the order and number of genes present within the individual I-DNAs, restriction enzyme digestions were performed with subsequent Southern blot analyses and these are described below. Additionally, genomic clones that spanned the I-DNAs were isolated and sequenced in the regions of the internal and external junction sites to complete and confirm the sequence composition of the I-DNAs determined from the Southern analyses.

b. Characterization (copy number, order and integrity) of the genes within the I-DNAs

Entire plasmid probe results. Southern blot analyses were performed on DNA isolated from leaf tissue of line MON 80100. Figures IV.13A and IV.13B show Southern blots of DNA isolated from line MON 80100 that was digested with a variety of restriction enzymes and probed with the plasmid probes PV-ZMGT10 (which contains the EPSPS CP4 and *gox* genes) and PV-ZMBK07 (which contains the *cryIA(b)* gene). Hybridization with the plasmid probes was performed in order to assess the overall genetic composition of the individual I-DNAs.

Hybridization with the plasmid probes allowed for the detection of multiple DNA fragments under all of the digestion conditions. Several digestions contained background bands (observed in the control sample cut with the same enzyme) and are noted on the left side of the identified band with asterisks within Figures IV.13A and

IV.13B. Background bands are defined as DNA fragments within the control and test samples that hybridize to the probe and therefore are not considered as resulting from the integrated DNA.

The plasmid probe was removed from the blots and then individual gene-specific probes were hybridized with these blots. This process allowed for the assessment of gene order and integrity. All of the fragments which hybridized to the entire plasmid probes were accounted for once all the cross-hybridization data, using individual gene specific probes, was evaluated. A schematic representation of the restriction digestion results when probed with the whole plasmid, accounting for all of the DNA fragments is shown in Figure IV.13C. The approximate sizes of the DNA fragments, released from the individual restriction enzyme digestions, are presented above each line within Figure IV.13C.

Integrity of the *cryIA(b)* genes. The PV-ZMBK07 probe was removed from the membrane shown in Figure IV.13B and then hybridized with either a full length *cryIA(b)* probe (3.5 Kb in length) or a less than full length *cryIA(b)* probe (prepared by PCR to approximately the first 900 bp of the *cryIA(b)* gene). The hybridization with the partial *cryIA(b)* probe allowed for an enhancement in signal intensity of the approximately 20 Kb DNA fragment in lanes 5 (Figure IV.14A versus IV.14B), the approximately 15 Kb band in lanes 8 (Figure IV.14A versus IV.14B), and the approximately 10 and 14 Kb bands in lanes 10 (Figure IV.14A versus IV.14B).

Evidence of a full length and a partial *cryIA(b)* gene was provided by the results of the simultaneous digestion with the restriction enzymes *Nco*I and *Eco*RI (Figure IV.14A, lane 7) which released two *cryIA(b)* gene fragments of approximate sizes 3.5 Kb and 2.9 Kb. The 3.5 Kb sized fragment is the expected size of the full length *cryIA(b)* gene released by the simultaneous digestion of *Eco*RI with *Nco*I restriction enzymes (Figure III.1). Since the 2.9 Kb *cryIA(b)* gene fragment was less than the expected size for the full length gene, this indicated that either or both of the *Eco*RI and *Nco*I sites were missing from the less than full length (partial) *cryIA(b)* gene. The presence of a full length and partial *cryIA(b)* gene supports the observation from the Southern blots in Figure IV.12, lane 4 which established that each of the two inserts contained a *cryIA(b)* gene or gene fragment.

In order to understand the composition of the partial gene, genomic clones containing the partial *cryIA(b)* gene were isolated and characterized. The clones established that the *Nco*I site was present (*i.e.*, the start ATG codon was intact) but that the *Eco*RI site at the

3' end of the gene was not present. Sequence analysis of the clones established the first 945 nucleotides of the *cryIA(b)* gene integrated into the corn genome and that a maximum open reading frame of 966 nucleotides is present within insert number 1, beginning at nucleotide number 1 of the *cryIA(b)* gene. The 966 bp open reading frame is for a protein that would contain amino acids 1-315 of the CryIA(b) protein and 7 amino acids of a non-CryIA(b) protein. The nucleotide sequence of the genomic DNA joined to the partial *cryIA(b)* gene showed no homology to any nucleotide sequence in GenBank (GenBank, 1991). Since amino acids 29-607 are necessary for insecticidal activity (Höfte and Whiteley, 1989), the partial *cryIA(b)* gene cannot produce an insecticidally active CryIA(b) protein. Western results (Figure IV.7) described above confirm that only the expected size CryIA(b) protein (and its protein degradation products) are detected from the integrated full length *cryIA(b)* gene in line MON 80100.

Various other restriction enzyme digestions were conducted and evaluated to determine the localization and order of the *cryIA(b)* genes within the I-DNAs. These will be discussed below. The results of the various hybridizations with the *cryIA(b)*, *gox*, CP4 and *nptII* probes were used to construct the schematics shown in Figure IV.14C which indicates the location of the *cryIA(b)* gene within the two inserts.

Integrity of the *gox* genes. Evidence of a full length and a partial *gox* gene was provided by the results of the simultaneous digestion of *Nco*I with *Eco*RI. Figure III.2 shows that a 1.3 Kb expected size fragment should be released from the simultaneous digestion with *Nco*I and *Eco*RI if a full length *gox* gene is present. Figure IV.15, lane 7 shows that the expected size 1.3 Kb DNA fragment and an approximately 6.0 Kb fragment hybridized with the *gox* specific probe. Since the expected size 1.3 Kb fragment was generated, this indicated that both *Eco*RI and *Nco*I restriction sites were present and the larger than expected 6.0 Kb fragment indicated that either or both of the *Eco*RI and *Nco*I sites were missing. The presence of a full length and a partial *gox* gene support the observation from the Southern blot in Figure IV.12, lane 6, which established that each of the two inserts contained a *gox* gene or gene fragment. The results of the various hybridizations with the *cryIA(b)*, *gox*, CP4 and *nptII* probes were used to construct the schematics shown in Figure IV.15B which indicates the location of the *gox* gene within the two inserts.

Integrity of the CP4 EPSPS genes. Evidence of a full length and partial CP4 EPSPS genes was observed. The *gox* probe was removed from the membrane shown in Figure IV.15 and the membrane was then hybridized with a CP4 EPSPS specific probe. Several bands were observed in the simultaneous *Nco*I with *Eco*RI digestion (Figure IV.16, lane 7), indicating that more than one CP4 EPSPS gene or gene fragments were present. The approximately 1 Kb band suggested that the *Nco*I site at nucleotide number 2290 and the *Eco*RI site at nucleotide number 3349 were present (Figure III.2) but did not demonstrate that a full length CP4 EPSPS gene was present. However, a *Bgl*II digestion released a 3.4 Kb DNA fragment which supported a full length CP4 EPSPS gene was present (Figure III.2) (data not shown).

From the sizes generated and cross-hybridization of DNA fragments with the CP4 EPSPS probe and the *cryIA(b)*, *gox* and *nptII* gene-specific probes and the sequence analysis of the internal junction sites within insert number 2, the order and number of CP4 EPSPS genes was established and is illustrated in Figure IV.16B. Figure IV.16B shows that 2 partial and one full length CP4 EPSPS gene are present in insert number 2.

Joining the partial *cryIA(b)* gene with the full length *gox* gene (insert number 1). The Southern blots in Figure IV.12 established that each I-DNA fragment contained a *cryIA(b)* and *gox* gene. Further restriction enzyme digestions and probings with the *cryIA(b)* and *gox* genes established that the less than full length *cryIA(b)* gene was located on the insert containing the full length *gox* gene (insert number 1) and the full length *cryIA(b)* gene was located on the same insert containing a less than full length *gox* gene (insert number 2). These conclusions are supported by the following data.

KpnI results. Figure IV.14A and IV.14B, lane 5 show a total of three hybridizing bands with the *cryIA(b)* probes (approximately 20, 4.3 and 3.1 Kb; the 20 Kb band is better visualized in Figure IV.14B, lane 5). When DNA from line MON 80100 was digested with *Kpn*I and probed with a *gox* gene probe, the same 20 Kb band was observed (Figure IV.15A, lane 5). This established that the 20 Kb DNA fragment was attributed to *Kpn*I cutting outside of insert number 1 (*i.e.*, within the corn genomic DNA). The 4.3 and 3.1 Kb bands were attributed to the generation of two fragments from the full length *cryIA(b)* gene since it is known to contain one internal *Kpn*I restriction site (Figure III.1). A diagrammatic representation of these results are shown in Figures IV.14C and IV.15B within the *Kpn*I results.

HindIII results. A comparison of lanes 8 in Figures IV.14B and IV.15A show a common 15 Kb hybridizing DNA fragment to the *cryIA(b)* and *gox* probes, respectively. Two fragments of approximate sizes 15 and 7.3 Kb hybridized to the *cryIA(b)* gene probe, Figure IV.14B, lane 8. The 15 Kb fragment is less intense than the 7.3 Kb fragment suggesting the 15 Kb fragment contained the partial (945 bp) *cryIA(b)* gene. When a Southern blot containing a *Hin*dIII digestion, was hybridized with the *gox* gene probe, two bands of approximate sizes 15 and 9 Kb were observed (Figure IV.15, lane 8). These results joined the partial *cryIA(b)* gene and a *gox* gene on a 15 Kb *Hin*dIII fragment, thereby supporting the *Kpn*I results described above. A diagrammatic representation of this result is shown in Figures IV.14C and IV.15B within the *Hin*dIII results.

BamHI results. The *Bam*HI digestion released two fragments of approximate sizes 10 and 14 Kb (of weak and strong signal intensities, respectively) that hybridized with the *cryIA(b)* gene probe (Figure IV.14B, lane 10). When the *Bam*HI digestion was probed with the *gox* gene, the 10 Kb fragment hybridized to it but the 14 Kb fragment did not. These results supported the *Kpn*I and *Hin*dIII digestions results which joined the partial *cryIA(b)* gene with a *gox* gene and are schematically illustrated in Figures IV.14C and IV.15B.

In all three digestion results, the *Kpn*I, *Hin*dIII and *Bam*HI restriction sites are located outside of insert number 1 (*i.e.*, within the genomic DNA) and this is reflected in Figures IV.14C, IV.15B and IV.16B. Additionally, since none of the three cross-hybridizing *cryIA(b)* and *gox* fragments (the 20 Kb fragment in *Kpn*I; the 15 Kb fragment in *Hin*dIII or the 10 Kb fragment in the *Bam*HI digestions) hybridized with the CP4 EPSPS gene probe (Figure IV.16, lanes 5, 8 and 10, respectively), this supported the observation made from Figure IV.12, lane 8 which indicated that insert number 1 did not contain a CP4 EPSPS gene.

In summary, the hybridization results of the *cryIA(b)* and *gox* gene specific probes with the *Kpn*I, *Hin*dIII and *Bam*HI digestions established that the partial *cryIA(b)* gene was joined with the *gox* gene on insert number 1. In order to determine the amount of the *gox* gene present on insert number 1, genomic clones were isolated and localized to this region of the I-DNA. Sequence analysis of the clone established that the entire *gox* gene was present through nucleotide number 6942 of the plasmid PV-ZMGT10 (Figure III.2 and Figure IV.2). Therefore, this established that insert number 1 contains a full length *gox* gene.

Joining the full length *cryIA(b)* gene with the insertion event containing the partial *gox* gene (insert number 2). Figures IV.14 and IV.15 (lanes 7) established that a full length and partial *cryIA(b)* and *gox* genes were present within the two I-DNAs. Since the results described above joined the partial *cryIA(b)* and full length *gox* genes on insert number 1, it was deduced that the full length *cryIA(b)* and partial *gox* genes must be located within insert number 2.

Joining and order of the full length *cryIA(b)*, CP4 EPSPS(s) and partial *gox* genes (insert number 2). Two restriction enzyme digestion results indicated that a region of CP4 EPSPS and *gox* gene fragments were juxtaposed. A comparison of the results from digesting the DNA with *Hin*dIII and probing with the *cryIA(b)*, *gox* and CP4 EPSPS gene probes showed that an approximately 9.0 Kb fragment did not hybridize to the *cryIA(b)* gene probe but did hybridize to both the *gox* and CP4 EPSPS gene probes (Figures IV.14, IV.15 and IV.16, lane 8). The results of the *Bam*HI digestion with the same three probings indicate that a 5.8 Kb fragment did not hybridize with the *cryIA(b)* probe but did hybridize to both of the *gox* and CP4 EPSPS probes. This established that the partial *gox* gene and a portion of a CP4 EPSPS gene were linked.

The *gox* gene was localized to one end of insert number 2 by looking for any common hybridization with a fragment that released it from the CP4 EPSPS genetic region. The *Nco*I digestion of the DNA indicated that two *gox* gene fragments were integrated into the corn genome since two fragments of approximate sizes 6.5 and 5.5 Kb were observed (Figure IV.15 lane 6). The less intense band at 6.5 Kb was labelled the partial *gox* gene and thereby assigned to insert number 2. The more intense band at 5.5 Kb was labelled the full length *gox* gene and thereby assigned to insert number 1, as defined above. Upon simultaneous digestion with *Eco*RI and *Nco*I, both fragments decreased in size. The 6.5 Kb fragment shifted to approximately 6.0 Kb while the 5.5 Kb band shifted to approximately 1.3 Kb (the expected size of the full length *gox* gene from known restriction sites within PV-ZMGT10, Figure III.2). The relative intensity of the two bands in the *NcoI* digest identified the partial *gox* gene as being contained on the 6.5 Kb fragment and therefore hybridization of this band or the 6.0 Kb band in the *NcoI/Eco*RI digestion was investigated for common hybridization to other probes; no common hybridizing bands were observed for the *cryIA(b)* or CP4 EPSPS gene probes, thereby establishing that the partial *gox* gene was at one end of insert number 2. If the *gox* gene had been internally located within insert number 2, a common hybridizing DNA fragment to the CP4 EPSPS or *cryIA(b)* gene probes would have been expected. These results are schematically illustrated in Figure IV.15B under the *Nco*I and *NcoI/Eco*RI digestion results.

Further information localizing the *gox* gene to one of the ends of insert number 2 was provided by sequencing a genomic clone localized to this region. Sequence analysis identified the *gox* gene to be broken at approximately nucleotide number 6127 (Figure III.2 and Figure IV.4) and juxtaposed to genomic DNA. This confirmed that the *gox* gene in insert number 2 is a partial gene.

The *cryIA(b)* gene was localized to the other end of insert number 2 containing the CP4 EPSPS and *gox* genes. In a separate Southern blot experiment, DNA from line MON 80100 was digested with *Eco*RI and simultaneously with *Eco*RI and *Spe*I and probed with the *cryIA(b)* gene probe (Figure IV.18, lanes 2 and 4). Upon digestion with *Eco*RI and probing with *cryIA(b)*, 5.0 and 7.8 Kb DNA fragments were observed. The 5.0 Kb band was previously localized to insert number 1 and therefore the 7.8 Kb DNA fragment was assigned to insert number 2. When *Spe*I was used simultaneously with *Eco*RI, the 7.8 Kb DNA fragment decreased in size to a 6.2 Kb size fragment. Since *Spe*I does not have a restriction site within either plasmid used to produce line MON 80100 (Figures III.1 and III.2), the DNA fragment size shift from 7.8 to 6.2, indicated that the *Spe*I site within the genomic DNA is internal (downstream) to one of the *Eco*RI sites located within the genomic DNA. Therefore the full length *cryIA(b)* gene was localized to one end and the *gox* gene to the other end on insert number 2. The order of the three genes in insert number 2 was thereby established to be *cryIA(b)* followed by CP4 EPSPS and then *gox*.

The far left end of insert number 2 (Figure IV.11B) contains a *Kpn*I and two *Hin*dIII sites between the *nptII* gene and the E35S promoter sequences. These restriction sites are not correspondingly located on the derived plasmid, PV-ZMBK07 (Figure III.1) but are located at the same relative position on plasmid PV-ZMGT10 (Figure III.2). These sites were placed at this position from the analysis of the *Kpn*I and *Hin*dIII restriction fragment sizes that were observed and presented above in Figure IV.14C under the *Kpn*I and *Hin*dIII results. These sites are presumed to have arisen from homologous recombination of the two plasmids in this area.

In summary, this work established that two I-DNAs are present in line MON 80100 derived from the plasmids PV-ZMBK07 and PV-ZMGT10. Full length and partial *cryIA(b)*, *gox*, CP4 EPSPS and *nptII* genes were observed and assigned to their respective I-DNAs. Multiple restriction enzyme digestions and subsequent hybridizations with the individual gene specific probes (*cryIA(b)*, *gox*, CP4 EPSPS and *nptII*) and DNA sequence analysis of genomic clones spanning the I-DNAs, established the order and integrity of the integrated genes shown schematically in Figures IV.10 and IV.11.

C. Segregation

Segregation data for the R1 plants (derived from selfing the original transformant, or R0 plant) and for the BC0F1 plants (derived from crossing the R0 with an inbred line) are presented in Table IV.1. The results are consistent with a single active insert segregating according to Mendelian expectations.

Table IV.1 Segregation data and analysis of progeny of insect protected corn line MON 80100

Generation	Actual	Expected	ChiSq
BC0F1[1]	14:6	10:10	2.450 *
R1[1]	27:13	30:10	0.833 *
BC0F2[1]	28:10	28.5:9.5	0.000 *
BC1F2 progeny [2]	101:244:139	121:242:121	6.000 #

[1] Data expressed as number of expressing plants: number of non-expressing plants based on European corn borer feeding assay

[2] Data expressed as number of homozygous expressing plants: number of segregants: number of homozygous susceptibles based on European corn borer feeding assay

*not significant at p = 0.05 (chi square = 3.84, 1 df)

not significant at p = 0.025 (chi square = 7.38, 2 df)

D. Stability of Gene Transfer

The stability of the genes within insect protected corn line MON 80100 has been demonstrated through five generations of crosses to one recurrent parent (B73) and 4 generations of crosses to a second, unrelated inbred (Mo17) (Table IV.2), and through three generations of selfing (BC1F4 data, Table IV.2). The Chi square test for the backcross to B73 did not deviate from expectations at p=0.05, and that to Mo17 did not deviate from expectation at p=0.01. The expectation among BC1F4 progeny based on the BC1F3 data showing a 3:1 segregation of 1/4 homozygous for the gene : 3/4 segregants and homozygote susceptibles was borne out by the non-significance of the Chi square test (Table IV.2).

To summarize the segregation and stability data (Tables IV.1 and IV.2), the data are consistent with a single active site of insertion of the *cryIA(b)* gene into genomic DNA of corn. The stability of this insertion has been demonstrated though five generations of crossing. The selfed data provide further verification of the stability of the insertion event.

Table IV.2 Segregation data for backcross derivatives of insect protected corn line MON 80100 in two unrelated inbred lines (B73 and Mo17). Values are ratios of plants that are positive or negative for the *B.t.k.* CryIA(b) protein as determined by ELISA.

Generation	Actual	Expected	ChiSq
BC4F1(B73) [1]	26:33	29.5:29.5	0.610 *
BC3F1(Mo17) [1]	40:20	30:30	6.017 #
BC1F4 [2]	1:5	1.5:4.5	0.000 *

[1] Data expressed as number of expressing plants: number of non-expressing plants based on *B.t.k.* CryIA(b)ELISA

[2] Data expressed as number of homozygous progeny: number of segregating or homozygous non-expressing progeny Btk gene PCR

* not significant at p = 0.05 (chi square = 3.84, 1 df)

not significant at p = 0.01 (chi square = 6.63, 1 df)

E. Conclusion

The corn line MON 80100 was produced by particle acceleration technology with the two plasmids PV-ZMBK07 and PV-ZMGT10 that contained the *cryIA(b)*, CP4 EPSPS, *gox* and *nptII* genes. Southern analyses established that two I-DNAs resulted from the particle acceleration process and that one insert, approximately 5.5 Kb, contains a partial *cryIA(b)* gene joined to a full length *gox* gene. The second insert, approximately 19 Kb, contains a full length *cryIA(b)* gene, a partial *gox* gene, two partial and one full length CP4 EPSPS genes, and a partial and a full length *nptII* gene. Western analyses established that only the expected size CryIA(b) and CP4 EPSPS proteins are produced and no detectable GOX protein is present in corn line MON 80100. The *nptII* gene is present in line MON 80100 under its bacterial promoter and its lack of detectable expression was confirmed by western analyses.

The analyses presented in this report enabled the generation of the detailed molecular maps shown in Figures IV.10 and IV.11 for the I-DNAs contained in the insect protected corn line MON 80100. The segregation and stability data are consistent with the stable introduction at a single active site of insertion of the *cryIA(b)* gene into genomic DNA of corn.

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Figure IV.3. 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
```

Figure IV.4. Deduced amino acid sequence of the GOX protein. Sequence includes the CTP1 transit peptide (amino acids 1-88 are the transit peptide).

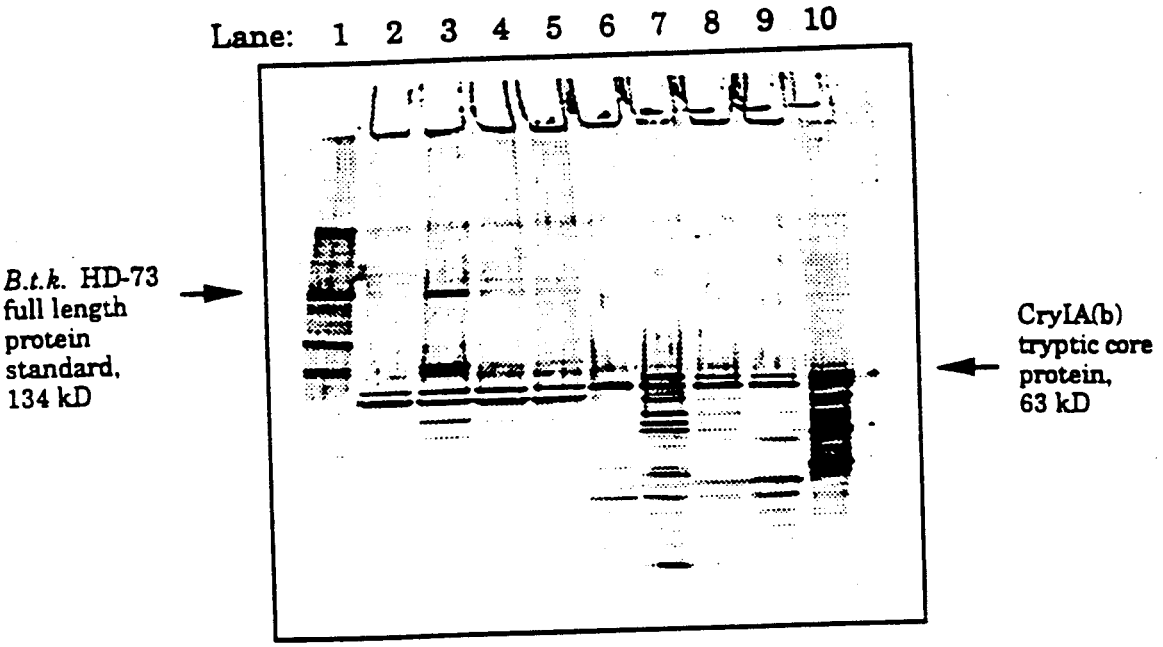
```
  1  MASSMLSSAT MVASPAQATM VAPFNGLKSS AAFPATRKAN NDITSITSNG
 51  GRVNCMQVWP PIGKKKFETL SYLPDLTDSG GRVNCMQAMA ENHKKVGIAG
101  AGIVGVCTAL MLQRRGFKVT LIDPNPPGEG ASFGNAGCFN GSSVVPMSMP
151  GNLTSVPKWL LDPMGPLSIR FSYFPTIMPW LIRFLLAGRP NKVKEQAKAL
201  RNLIKSTVPL IKSLAEEADA SHLIRHEGHL TVYRGEADFA KDRGGWELRR
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
```

Figure IV.5. 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
```

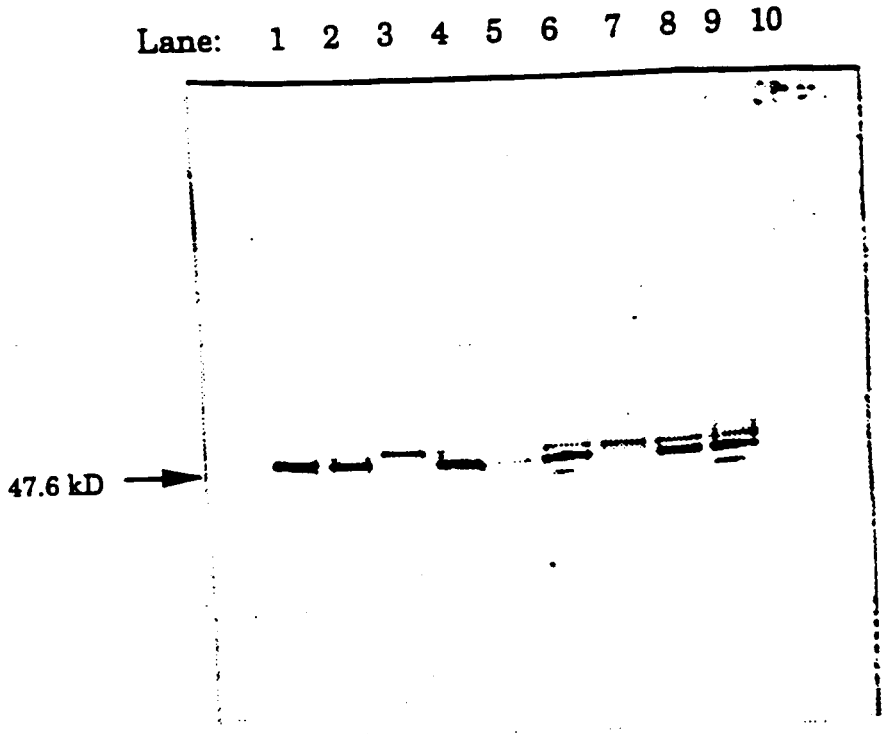
Figure IV.6. Deduced amino acid sequence of the NPTII protein.

```
  1  MIEQDGLHAG SPAAWVERLF GYDWAQQTIG CSDAAVFRLS AQGRPVLFVK
 51  TDLSGALNEL QDEAARLSWL ATTGVPCAAV LDVVTEAGRD WLLLGEVPGQ
101  DLLSSHLAPA EKVSIMADAM RRLHTLDPAT CPFDHQAKHR IERARTRMEA
151  GLVDQDDLDE EHQGLAPAEL FARLKARMPD GEDLVVTHGD ACLPNIMVEN
201  GRFSGFIDCG RLGVADRYQD IALATRDIAE ELGGEWADRF LVLYGIAAPD
251  SQRIAFYRLL DEFF
```



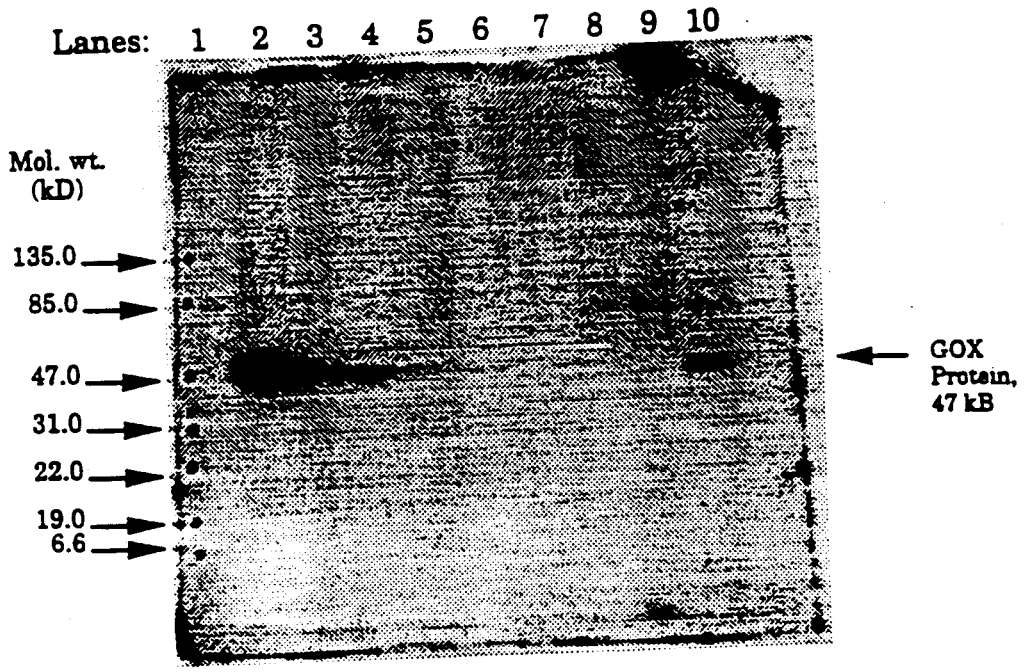
Lane	Description
1	10 ng of *B.t.k.* HD-73 full length standard from *E. coli*
2	MON 80080, control, corn leaf extract, 47.32 µg protein
3	MON 80200 corn leaf extract, 45.36 µg protein
4	MON 80100 corn leaf extract, 82.04 µg protein
5	MON 80400 corn leaf extract, 45.36 µg protein
6	MON 80080, control, corn leaf extract, trypsinized, 17.08 µg protein
7	MON 80200 corn leaf extract, trypsinized, 9.52 µg protein
8	MON 80100 corn leaf extract, trypsinized, 12.04 µg protein
9	MON 80400 corn leaf extract, trypsinized, 11.06 µg protein
10	10 ng of CryIA(b) standard from *E. coli*, trypsinized

Figure IV.7. Western blot analysis of *B.t.k.* HD-1 CryIA(b) proteins in corn tissue extracts. All three insect protected corn lines evaluated, MON 80200, MON 80100 and MON 80400 were produced using the same *cryIA(b)* gene. The multiple bands are *B.t.k.* protein degradation products.



Lane	Description
1	10 ng of purified CP4 EPSPS produced in *E. coli*,
2	10 ng of purified CP4 EPSPS produced in ECB protected corn line 523-06-1
3	0.5 µg of a whole plant protein extract made from the control line, MON 80080
4	0.2 µg of a whole plant protein extract made from ECB protected corn line 523-06-1
5	0.5 µg of a whole plant extract made from ECB protected corn line MON 80100
6	10 ng of purified CP4 EPSPS produced in *E. coli* spiked into 0.5 µg of a whole plant protein extract made from the control line, MON 80080
7	2 µg of a seed protein extract from the control line, MON 80080
8	2 µg of a seed protein extract from ECB protected corn line MON 80100
9	10 ng of purified CP4 EPSPS produced in *E. coli* spiked in 2 µg of a seed protein extract from MON 80080, the control line
10	Sigma Broad Range Molecular Weight Markers

Figure IV.8. Western blot analysis of CP4 EPSPS protein in corn tissue extracts. Both corn lines MON 80100 and line 523-06-1 were produced with the same CP4 EPSPS gene.



Lane	Description
1	Sigma rainbow markers (spotted after transfer)
2	10.0 ng of *E. coli*-produced GOX spiked into 30 µg of control corn (MON 81800) line protein extract
3	5.0 ng of *E. coli*-produced GOX spiked into 30 µgof control corn (MON 81800) line protein extract
4	2.5 ng of *E. coli*-produced GOX spiked into 30 µg of control corn (MON 81800) line protein extract
5	1.0 ng of *E. coli*-produced GOX spiked into 30 µg of control corn (MON 81800) line protein extract
6	0.5 ng of *E. coli*-produced GOX spiked into 30 µg of control corn (MON 81800) line protein extract
7	0.1 ng of *E. coli*-produced GOX spiked into 30 µg of control corn (MON 81800) line protien extract
8	30 µg of control line (MON 81800) protein extract
9	30 µg of ECB protected corn line (MON 80100) protein extract
10	3.0 µg of ECB protected corn line (MON 80200) protein extract

Figure IV.9. Western blot analysis of GOX protein in corn tissue extracts. Both corn lines MON 80100 and MON 80200 were produced using the same *gox* gene. The tissue source in lanes 8, 9, and 10 was young leaf.

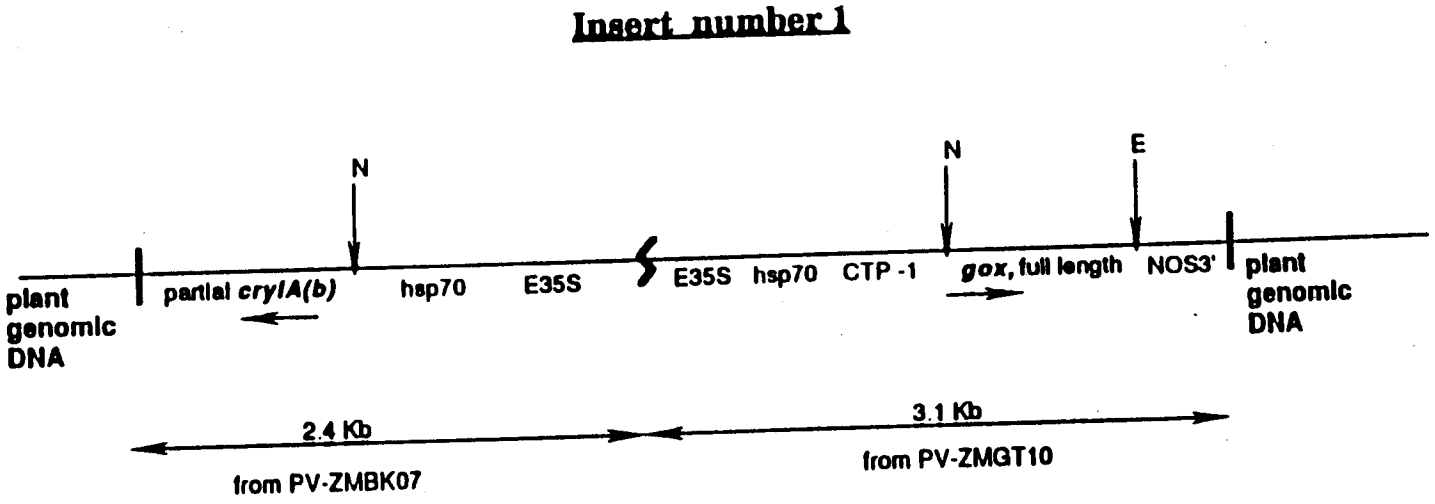


Figure IV.10. Schematic illustration of insert number 1. The broken vertical line in the middle of the figure denotes a plasmid-plasmid internal junction. The vertical arrows denote the locations of internal restriction sites. The letters over the arrows are abbreviations for the restriction enzymes: N=*Nco*I and E=*Eco*RI. The horizontal arrows delineate the region and respective sizes of the plasmid composition of the insert. The size of this insert is approximately 5.5 Kb

Insert number 2

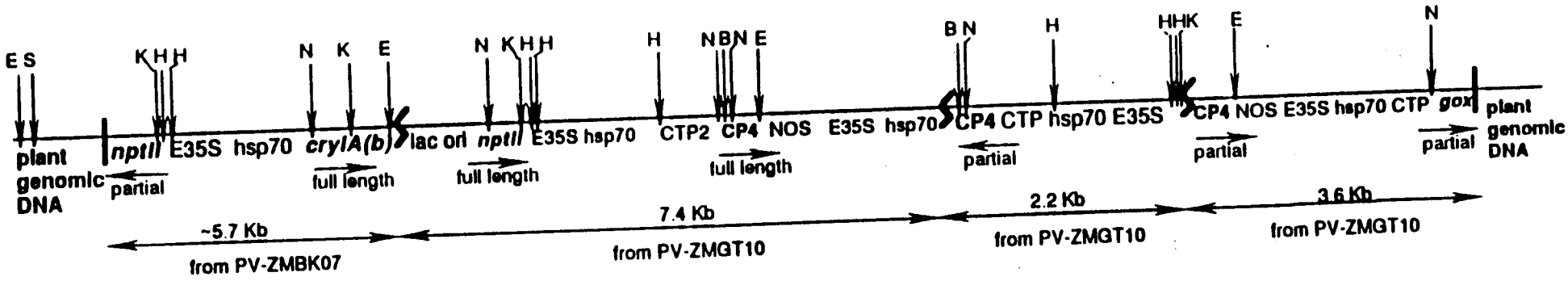


Figure IV.11. Schematic illustration of insert number 2. The broken vertical lines in the figure denote plasmid-plasmid junctions. The vertical arrows denote the locations of internal restriction sites. The letters over the arrows are abbreviations for the restriction enzymes: E=*Eco*RI, S=*Spe*I, K=*Kpn*I, H=*Hin*dIII, N=*Nco*I, B=*Bam*HI. The horizontal arrows delineate the region and respective sizes of the plasmid composition of the insert. The size of this insert is approximately 19 Kb .

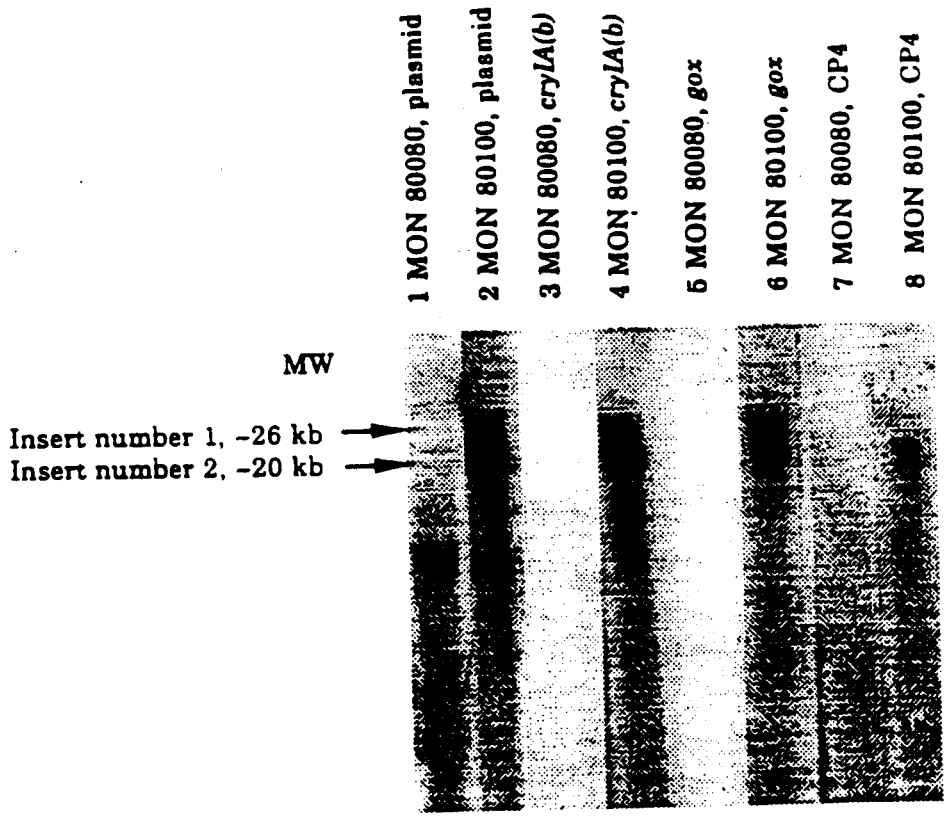


FIGURE IV.12. Southern blot analysis of DNA isolated from line MON 80100 for insert number assessment. The lanes contain either approximately 10 µg of control DNA isolated from MON 80080 or 10 µg of DNA isolated from line MON 80100 digested with *Nde*I and hybridized with the probes listed above each lane. The plasmid probe is a mixture of PV-ZMGT10 and PV-ZMBK07.

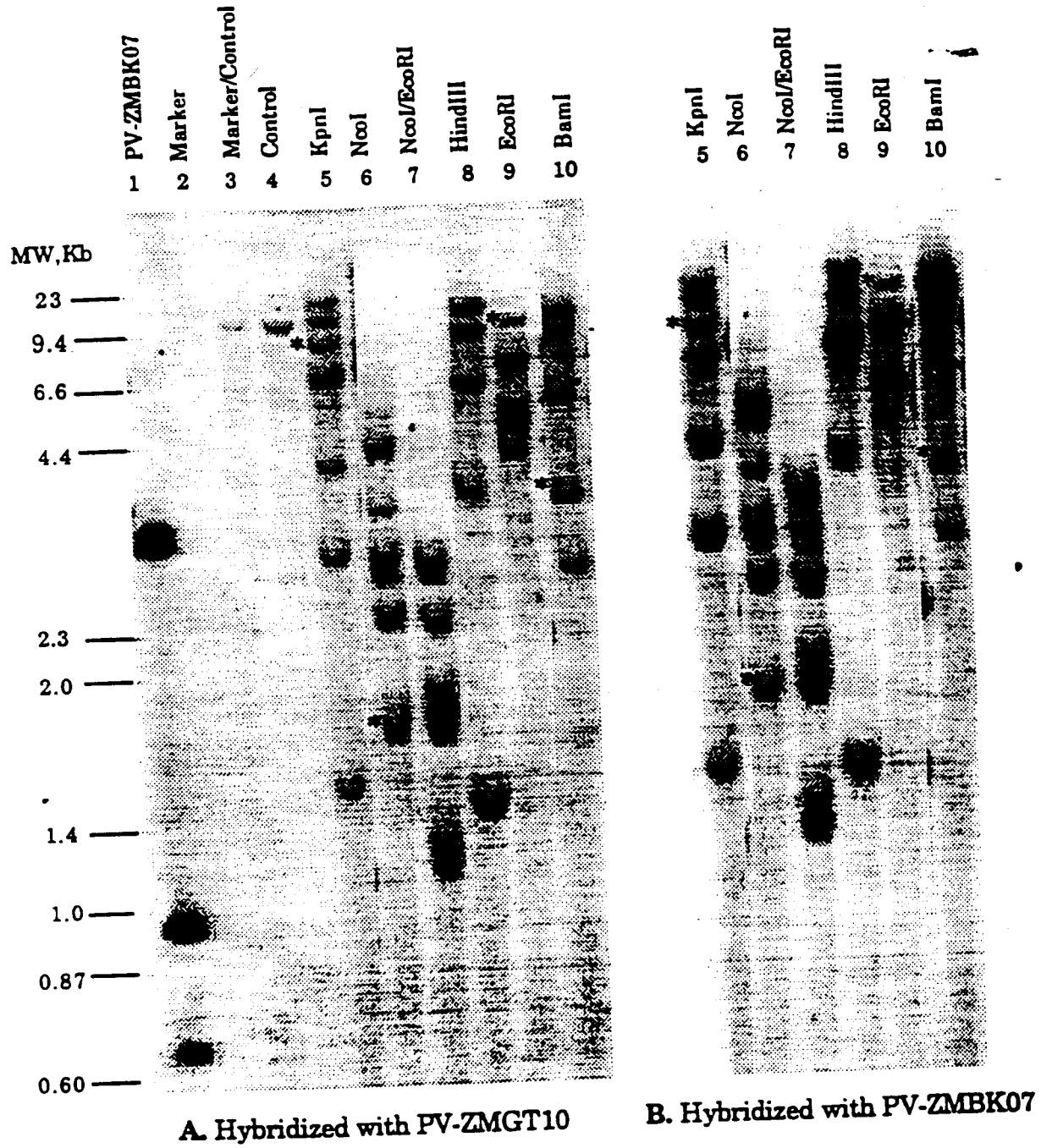


FIGURE IV.13. Southern blot analysis of DNA isolated from line MON 80100 and hybridized with whole plasmid probes and a schematic illustration of the results. A. Lane 1 contains 50 pg of PV-ZMBK07 digested with *Pst*I. Lane 2 contains molecular weight standards. Lane 3 contains molecular weight standards spiked into 10 µg of control DNA isolated from line MON 80080 that was digested with *Eco*RI. Lane 4 contains 10 µg of control DNA from line MON 80080 digested with *Eco*RI. Lanes 5-10 each contain approximately 10 µg of DNA from line MON 80100 digested with the enzymes listed above each lane. B. Southern blot analysis of line MON 80100 probed with PV-ZMBK07. Lane designations are the same as those in Figure IV.13A. Asterisks denote background bands.

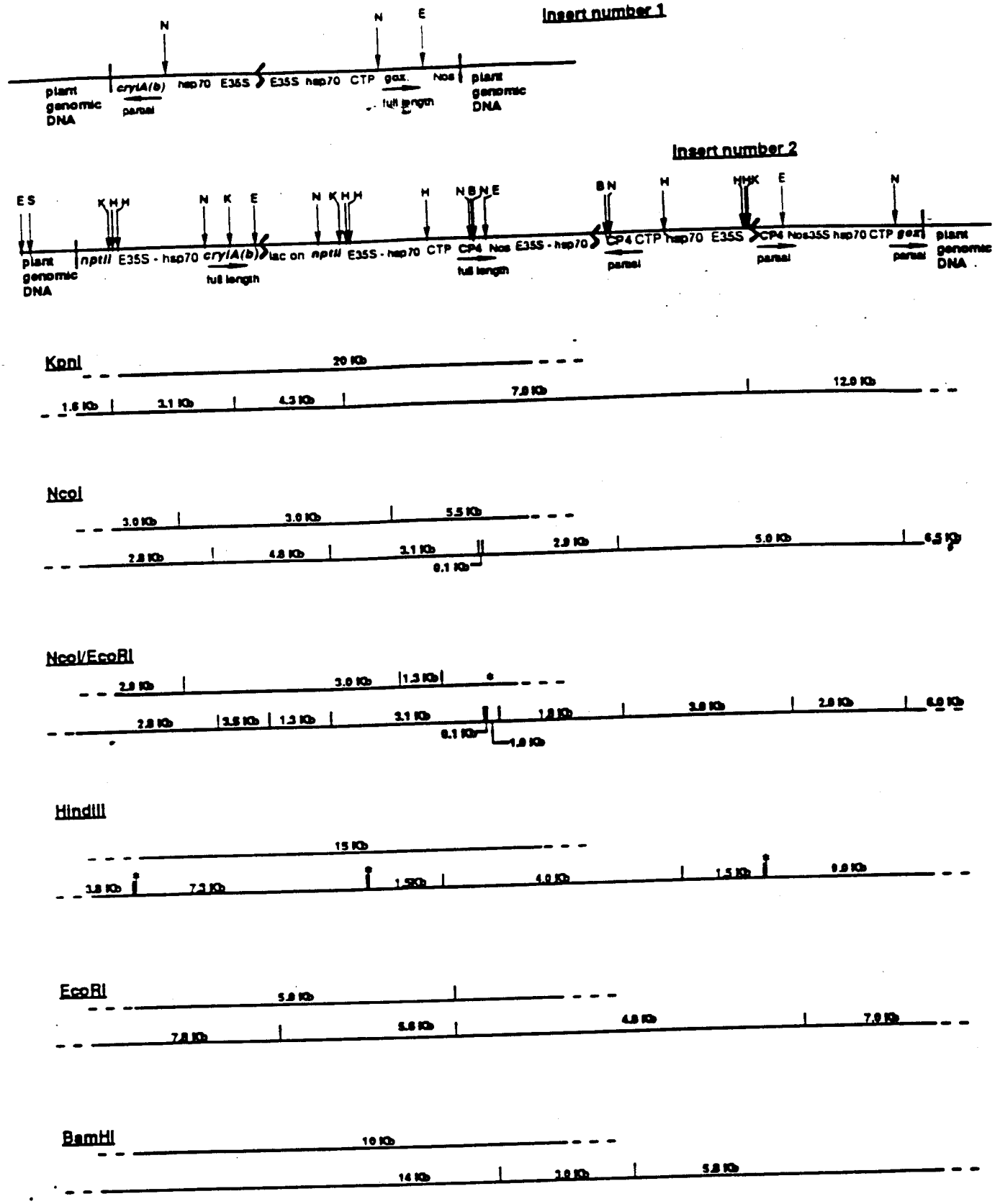
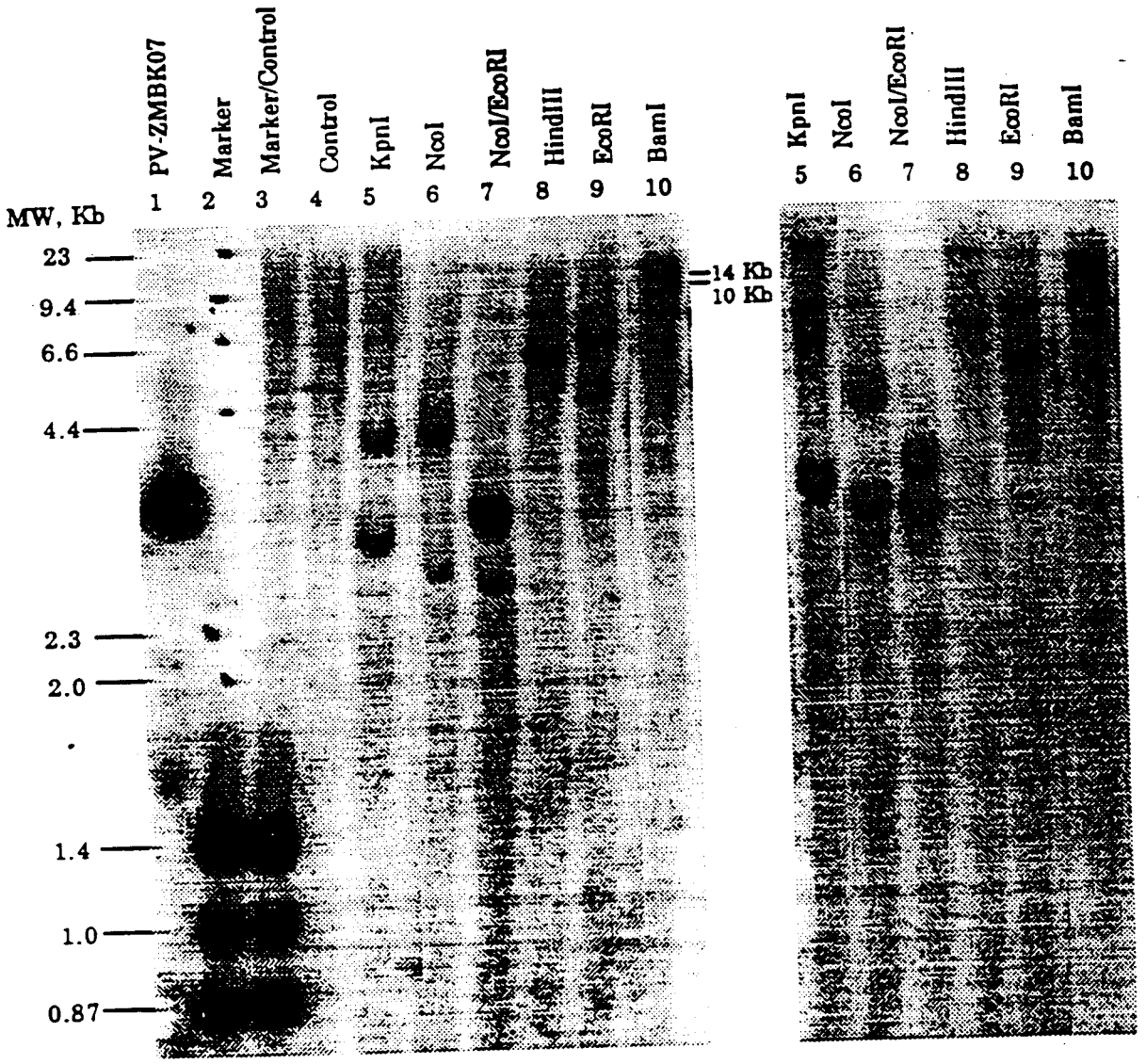


Figure IV.13C. A schematic illustration of the DNA fragments that hybridize to the plasmid probes. Each set of restriction digestions contain sizes generated for insert number 1 (top) and insert number 2 (bottom). Sizes given are estimates. Asterisks indicate fragments not detected (assumed to be too small). Vertical lines indicate the locations of restriction sites within the I-DNA. The horizontal arrows indicate the orientation of the genes in the 5' to 3' direction. The vertical arrows indicate the locations of the restriction sites within the I-DNA and the abbreviations are as follows: E=*Eco*RI, S=*Spe*I, K=*Kpn*I, H=*Hin*dIII, N=*Nco*I, B=*Bam*HI. Not to scale.



A. Hybridized with a 3.5 Kb *cryIA(b)* probe.

B. Hybridized with a 0.9 Kb *cryIA(b)* probe.

FIGURE IV.14. Southern blot analysis of DNA isolated from line MON 80100 hybridized with *cryIA(b)* probes and a schematic illustration of the results. A. Lane 1 contains 50 pg of PV-ZMBK07 digested with *Pst*I. Lane 2 contains molecular weight standards. Lane 3 contains molecular weight standards spiked into 10 µg of control DNA isolated from line MON 80080 digested with *Eco*RI. Lane 4 contains 10 µg of control DNA isolated from line MON 80080 digested with *Eco*RI. Lanes 5-10 each contain 10 µg of DNA isolated from line MON 80100 digested with the enzymes listed above each lane. **B.** Southern blot analysis of line MON 80100 probed with a 0.9 Kb *cryIA(b)* probe. Lane designations are as in Figure IV.14A.

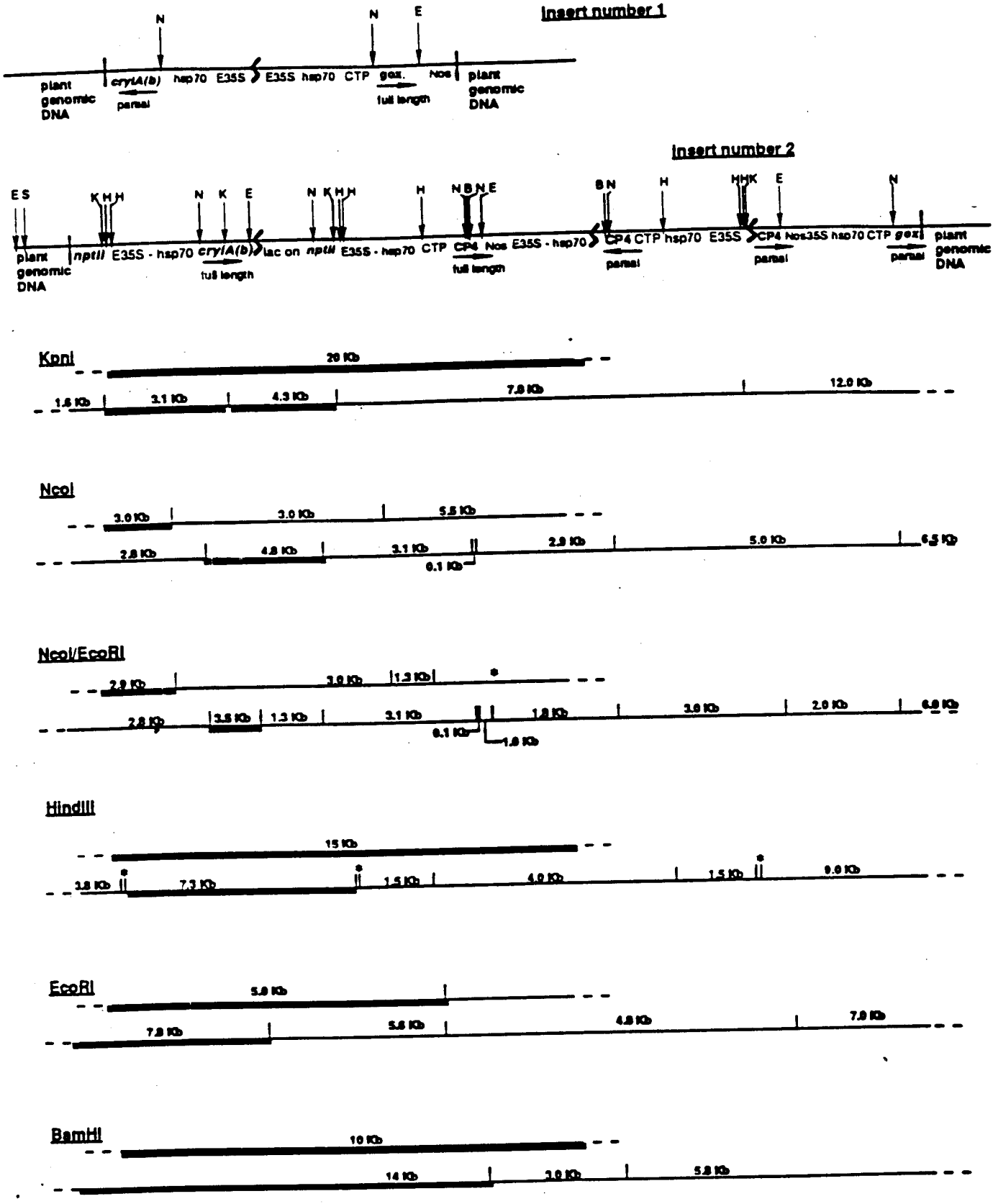


Figure IV.14C. A schematic illustration of the DNA fragments that hybridize to the *cryIA(b)* probes. Each set of restriction digestions contain sizes generated for insert number 1 (top) and insert number 2 (bottom). Sizes given are estimates. Asterisks indicate fragments not detected (assumed to be too small). Vertical lines indicate the locations of restriction sites within the I-DNA. The horizontal arrows indicate the orientation of the genes in the 5' to 3' direction. The vertical arrows indicate the locations of the restriction sites within the I-DNA and the abbreviations are as follows: E=*Eco*RI, S=*Spe*I, K=*Kpn*I, H=*Hind*III, N=*Nco*I, B=*Bam*HI. Not to scale.

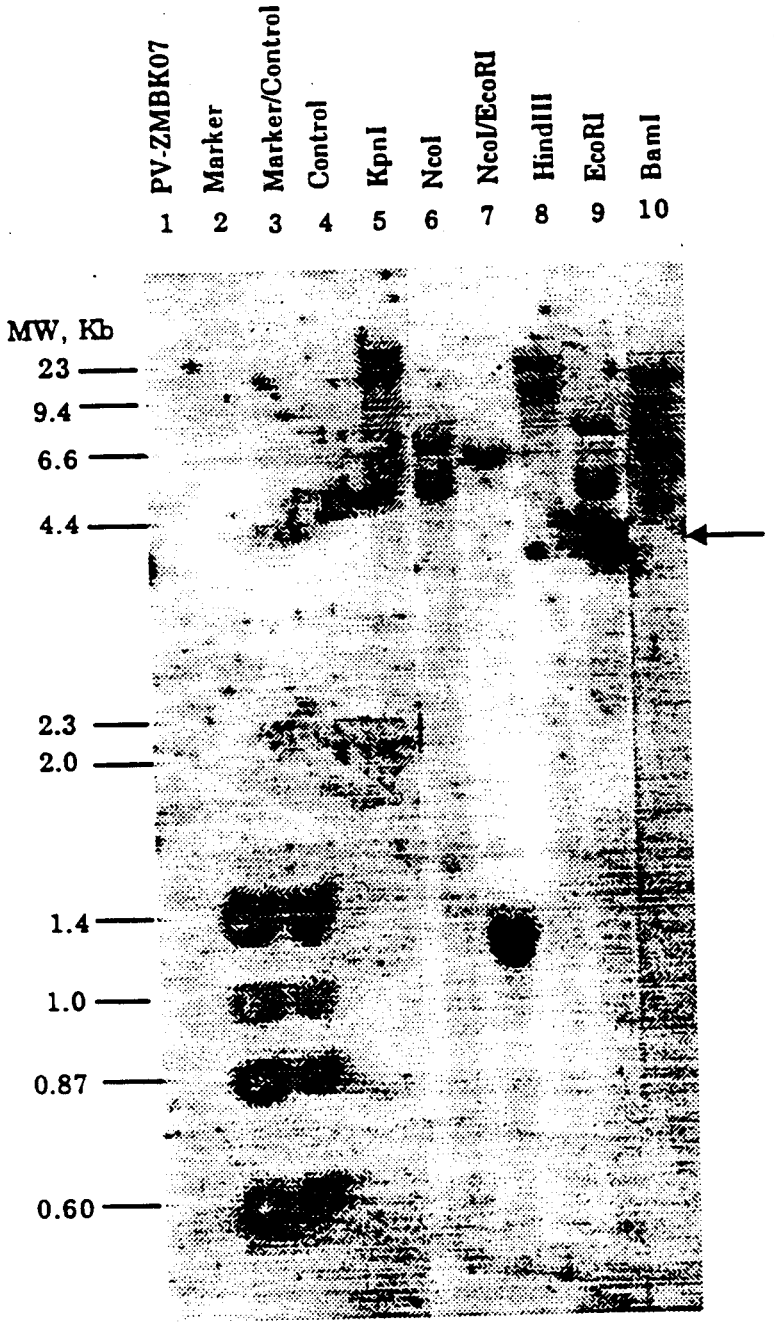


FIGURE IV.15. Southern blot analysis of DNA isolated from line MON 80100 hybridized with a *gox* probe and a schematic illustration of the results. A. Lane 1 contains 50 pg of PV-ZMBK07 digested with *Pst*I. Lane 2 contains molecular weight standards. Lane 3 contains molecular weight standards spiked into 10 µg of control DNA isolated from line MON 80080 digested with *Eco*RI. Lane 4 contains 10 µg of control DNA isolated from line MON 80080 digested with *Eco*RI. Lanes 5-10 each contain 10 µg of DNA isolated from line MON 80100 digested with the enzymes listed above each lane. The arrow indicates non-specific hybridization.

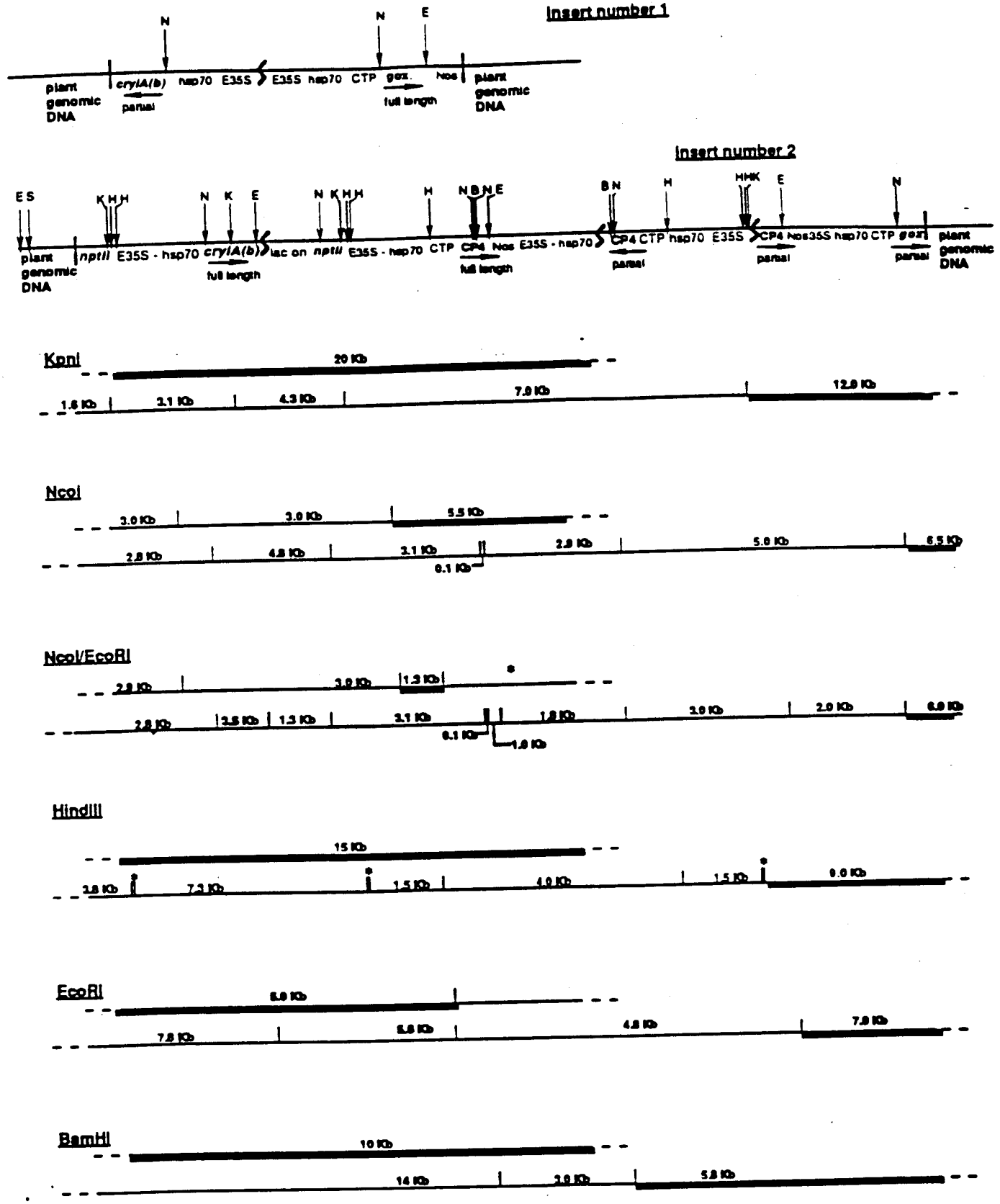


Figure IV.15B. A schematic illustration of the DNA fragments that hybridize to the *gox* probe. Each set of restriction digestions contain sizes generated for insert number 1 (top) and insert number 2 (bottom). Sizes given are estimates. Asterisks indicate fragments not detected (assumed to be too small). Vertical lines indicate the locations of restriction sites within the I-DNA. The horizontal arrows indicate the orientation of the genes in the 5' to 3' direction. The vertical arrows indicate the locations of the restriction sites within the I-DNA and the abbreviations are as follows: E=*EcoRI*, S=*SpeI*, K=*KpnI*, H=*HindIII*, N=*NcoI*, B=*BamHI*. Not to scale.

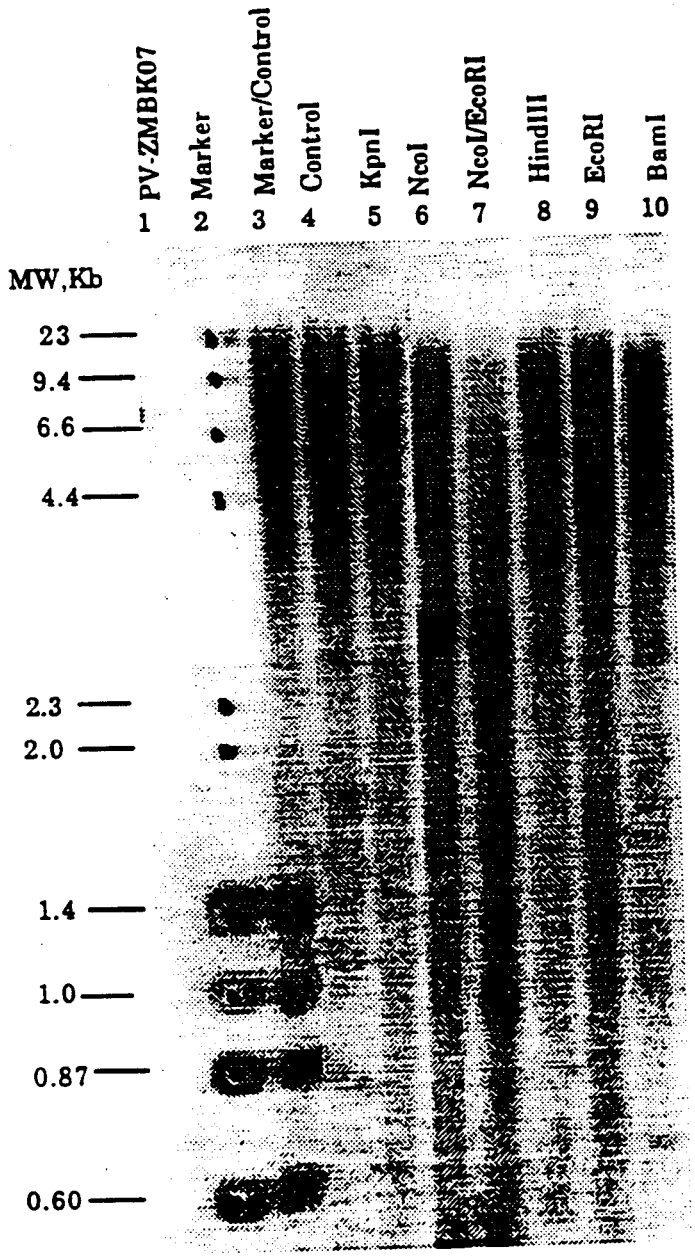


FIGURE IV.16. Southern blot analysis of DNA isolated from line MON 80100 hybridized with a CP4 EPSPS probe and a schematic illustration of the results. A. Lane 1 contains 50 pg of PV-ZMBK07 digested with *Pst*I. Lane 2 contains molecular weight standards. Lane 3 contains molecular weight standards spiked into 10 µg of control DNA MON 80080 digested with *Eco*RI. Lane 4 contains 10 µg of control DNA, MON 80080, digested with *Eco*RI. Lanes 5-10 each contain 10 µg of DNA from line MON 80100 digested with the enzymes listed above each lane.

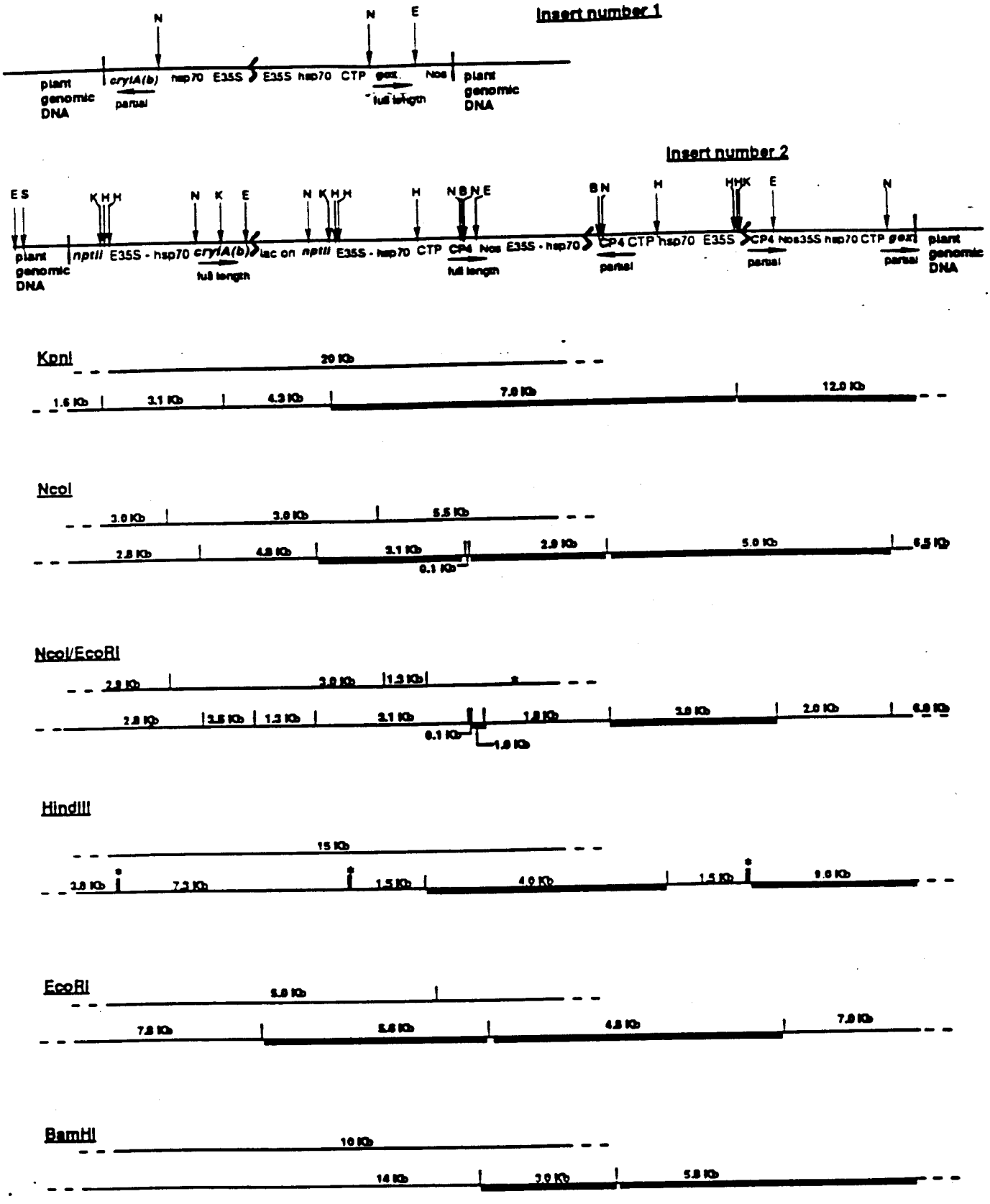


Figure IV.16B. A schematic illustration of the DNA fragments that hybridize to the CP4 EPSPS probe. Each set of restriction digestions contain sizes generated for insert number 1 (top) and insert number 2 (bottom). Sizes given are estimates. Asterisks indicate fragments not detected (either because of their size or not enough CP4 EPSPS probe homology was present within the fragment). Vertical lines indicate the locations of restriction sites within the I-DNA. The horizontal arrows indicate the orientation of the genes in the 5' to 3' direction. The vertical arrows indicate the locations of the restriction sites within the I-DNA and the abbreviations are as follows: E=*Eco*RI, S=*Spe*I, K=*Kpn*I, H=*Hin*dIII, N=*Nco*I, B=*Bam*HI. Not to scale.

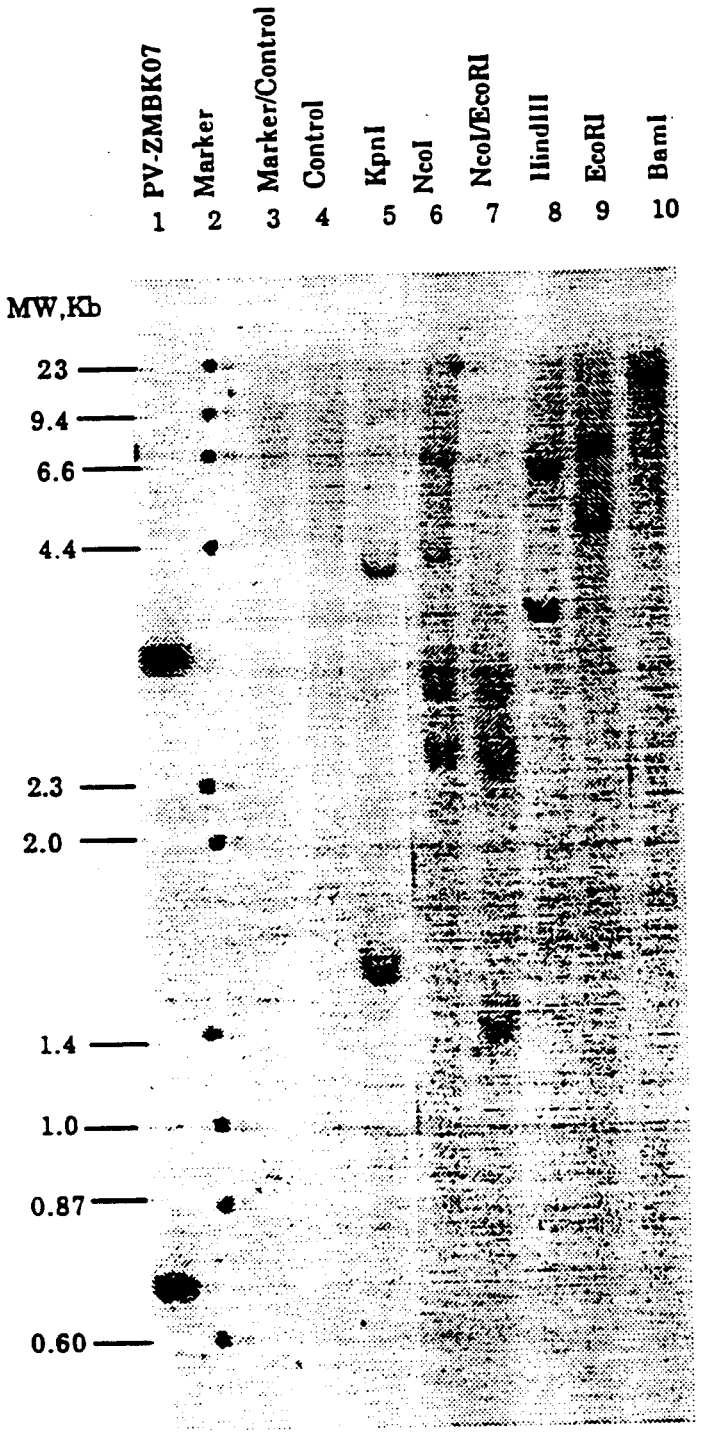


FIGURE IV.17. Southern blot analysis of DNA isolated from line MON 80100 hybridized with a *nptII* probe and a schematic illustration of the results. A. Lane 1 contains 50 pg of PV-ZMBK07 digested with *Pst*I. Lane 2 contains molecular weight standards. Lane 3 contains molecular weight standards spiked into 10 µg of control DNA isolated from line MON 80080 digested with *Eco*RI. Lane 4 contains 10 µg of control DNA isolated from line MON 80080 digested with *Eco*RI. Lanes 5-10 each contain approximately 10 µg of DNA isolated from line MON 80100 digested with the enzymes listed above each lane.

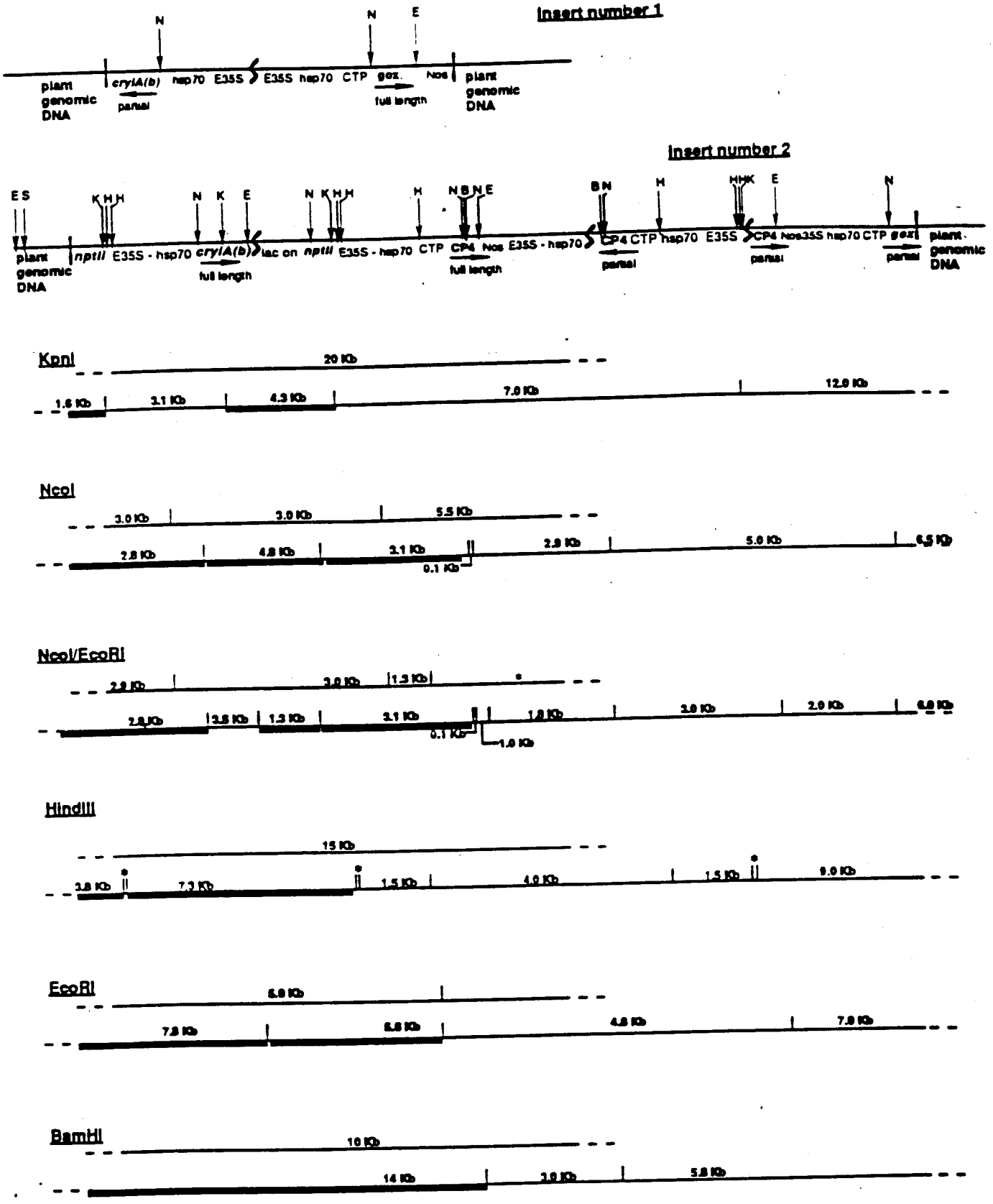


Figure IV.17B. A schematic illustration of the DNA fragments that hybridize to the *nptII* probe. Each set of restriction digestions contain sizes generated for insert number 1 (top) and insert number 2 (bottom). Sizes given are estimates. Asterisks indicate fragments not detected (assumed to be too small). Vertical lines indicate the locations of restriction sites within the I-DNA. The horizontal arrows indicate the orientation of the genes in the 5' to 3' direction. The vertical arrows indicate the locations of the restriction sites within the I-DNA and the abbreviations are as follows: E=*EcoRI*, S=*SpeI*, K=*KpnI*, H=*HindIII*, N=*NcoI*, B=*BamHI*. Not to scale.

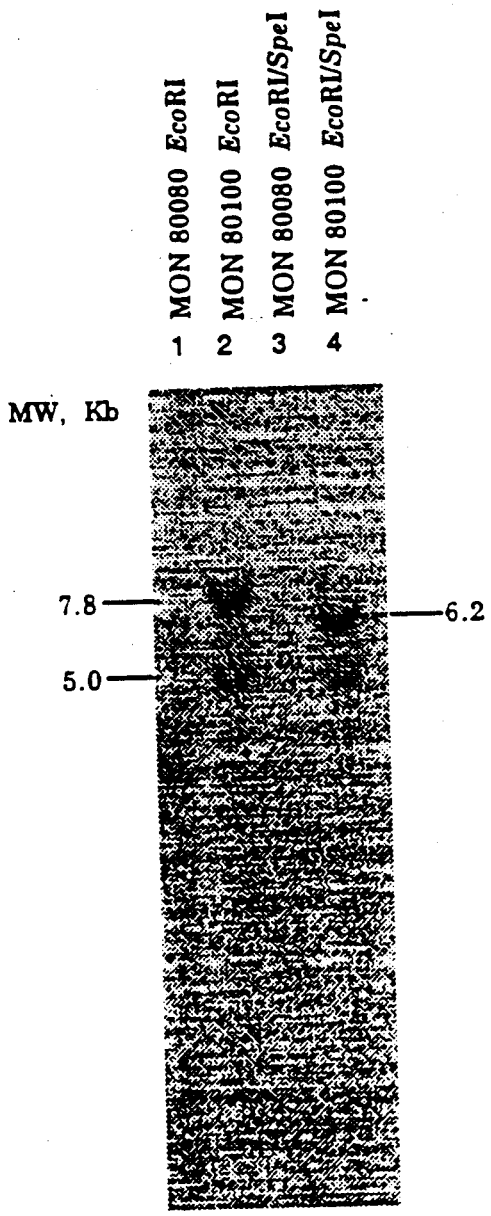


FIGURE IV.18. Southern blot analysis of DNA isolated from line MON 80100 digested with *Eco*RI vs. *Eco*RI/*Spe*I and hybridized with a *cryIA(b)* probe. Both control DNA and MON 80100 were digested in 10µg amounts with the enzymes listed above each lane and hybridized with a *cryIA(b)* probe.

Part V. Detailed Description of the Phenotype of Insect Protected Corn Line MON 80100

Introduction

Data and information supplied in this Petition for Determination of Non-Regulated Status demonstrate that insect protected corn line MON 80100 is substantially equivalent to non-modified corn, except for the inserted genetic sequences, the expressed proteins [CryIA(b) protein and CP4 EPSPS enzyme], and the ability of the plant to resist damage from certain Lepidopteran insects including European corn borer. The information supplied in this section and referenced from other sections of this petition will demonstrate that the modified, insect protected corn line MON 80100 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 inserted proteins, and the lack of any deletarious environmental fate/effects.

A variety of studies were conducted to characterize the unique traits of the modified corn line and to establish that insect protected corn line MON 80100 is substantially equivalent to non-modified corn. The inserted genetic material in MON 80100 were described in the previous sections (Part III and IV). The other characteristic unique to MON 80100, expression of the CryIA(b) and CP4 EPSPS proteins, is described in this section. Other investigations included:

- safety assessment of the *B.t.k.* CryIA(b) protein to non-target insects
- the environmental fate of the *B.t.k.* CryIA(b) protein
- the potential for outcrossing and weediness
- laboratory and field germination results
- the disease and pest susceptibility of MON 80100
- yield characteristics
- the comparison of MON 80100 and its parental control, on the basis of composition and quality 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 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 as 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 *et al*, 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. Expression Levels of the CryIA(b), CP4 EPSPS, GOX, and NPTII Proteins

As described in Part III, insect protected corn line MON 80100 has been modified to express a protein from *Bacillus thuringiensis* subsp. *kurstaki* strain CryIA(b) (Höfte and Whitely, 1989). This protein, CryIA(b) has insecticidal activity against the European corn borer (ECB), an economically damaging corn insect pest. In addition to the *cryIA(b)* gene, genes encoding CP4 EPSPS (Padgette *et al*, 1993), GOX (Padgette *et al*, 1994), and NPTII are also present. The CP4 EPSPS and GOX genes are present to enable selection of cells in tissue culture that contain the *cryIA(b)* gene. The corn transformation vectors used to produce corn line MON 80100 included the gene cassette containing a bacterial specific promoter and coding region for NPTII. NPTII allows selection of bacteria containing the vector in media containing kanamycin. The *npt*II gene is under the control of a bacterial-specific promoter and, therefore, is not expected to produce the NPTII protein in plant cells. The control line, MON 80080, has background genetics representative of the test line, but has not been genetically modified and therefore, does not express the CryIA(b), CP4 EPSPS, GOX, or NPTII proteins.

Levels of the expressed proteins were evaluated in young leaf and seed tissues collected from five field locations during the 1993 growing season using an Enzyme Linked Immuno-Sorbent Assay (ELISA) (Harlow and Lane, 1988)

and western blot methods (Matsudaira, 1987). The five field sites established and conducted under GLP were as follows: Jerseyville, Illinois; Monmouth, Illinois; Johnston, Iowa; Winterset, Iowa; Windfall, Indiana; and York, Nebraska. The approximate expression levels are shown in the following table.

Table V.1 Summary of specific protein levels measured in MON 80100 tissues[1].

Protein	Leaf	Seed	Whole plant[4]	Pollen[5]
	-µg/g fresh weight-			
CryIA(b)	1.3	0.57	1.77	N.D.[2]
CP4 EPSPS	1.85	4.11	0.57	N.A.[3]
GOX	N.D.	N.D.	N.D.	N.A.
NPTII	N.D.	N.D.	N.A.	N.A.

1: Values are means calculated across five sites from mean values calculated from the analysis of 3-4 replicate samples per site.
2: Not detected
3: Not analyzed
4: Values are means calculated from four replicate samples from one site.
5: Determination from duplicate analysis of one pollen sample from one site.

As seen above, expression levels of CryIA(b) and CP4 EPSPS proteins are low in corn leaf, seed and whole plant tissues. CryIA(b) protein was not detectable in pollen.

GOX protein was not detectable in corn leaf, seed and whole plant tissue when assayed by sensitive and specific ELISA and this result was confirmed by western blot analysis.

As expected, the NPTII protein, whose expression is driven by a bacterial promoter, was not detected in corn tissue.

D. 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 beetles

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).

Insect protected corn line MON 80100 also encodes the enzyme CP4 EPSPS as discussed above. EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants (including corn) and microorganisms (Levin and Sprinson, 1964), 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 *et al*, 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. EPSPS is considered to be ubiquitous in nature since it is present in all plants and microorganisms. Therefore, all organisms which presently feed on plants and/or microbes, historically have been exposed to EPSPS.

2. Non-Target Wildlife and Fish

A study was conducted to assess the wholesomeness of insect protected corn meal fed to quail since birds may feed on corn left in the field after harvest. No mortality occurred in birds fed up to 10% w/w (nominal 100,000 ppm) raw corn seed meal in the diet. This feeding level approximates consumption of 138 corn seeds/kg body weight/ bird/day. The no-observed effect level was considered to be greater than 10% w/w. Based on the parameters measured, the wholesomeness of meal from insect protected corn seed was comparable to that of the parental line when fed in the diet to quail (Campbell and Beavers, 1994).

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 CryIA(b) protein as expressed in corn.

3. Impact on Endangered Species

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

E. 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 corn line MON 80100 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 as 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).

F. 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 I.A 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 and an extensive review of literature, 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. except for special plantings, 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.

   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; Mangelsdorf, 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 Insect Protected 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 I.A). 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)* or CP4 EPSPS 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. Further, the CP4 EPSPS gene was isolated from an *Agrobacterium* sp., 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.

G. Laboratory and Field Germination Results

Laboratory and field germination studies were initiated in 1994 to compare the survival characteristics of the insect protected corn line MON 80100 to its parental control. Seed for MON 80100 and its parental control were produced in the same field location established under GLP (USDA Notification 94-105-14N). The seed was tested to determine the germination rate. A standard germination test was performed using seed germination trays and paper in greenhouse conditions, and evaluated after five days. Results for the two lines in the germination test were not significantly different (Table V.2). No differences were observed in susceptibility of the seed tested in the laboratory to disease (*Fusarium* sp.) and rice weevil (*Sitophilus oryzae*) and maize weevil (*S. zeamais*).

Table V.2 **Laboratory germination results for insect protected corn line MON 80100 and its parental control.** Conducted at Monsanto Life Sciences Center, Chesterfield, MO. Standard germination test (5 day). Daytime temperature: 86° F, nightime temperature: 78° F. Photoperiod: 14 hr. day/10 hr. night. Samples consisted of 25 seed from 15 individual corn ears.

Line	Sample number	Percent germination
MON 80100	15	97.6%a
Control	15	97.1%a

(Means followed by the same letter do not significantly differ as determined by analysis of variance procedures)

Seed and intact corn ears were planted at two timings (October/November) in overwintering trials at Monsanto research farm locations in Jersey and Warren Counties in Illinois. These field trials were established in 1994. Preliminary data (fall germination results) from the October planting indicate no differences between MON 80100 and the parental control.

H. Disease and Pest Susceptibilities

Insect protected corn line MON 80100 has been tested in the United States in sixty two locations in thirteen states since the first field trial in 1992. Detailed monitoring for the disease and insect susceptibility of this line versus non-transgenic plants was performed in 1992, 1993, and 1994 at the sites listed in Table V.5. Example monitoring forms are provided in Appendix III. No differences in agronomic quality, disease, or insect susceptibility other than European corn borer control were detected between MON 80100 and non-transgenic plants. Diseases observed included northern leaf blight (*Exserohilum turcicum),* southern leaf blight (*Bipolaris maydis*), bacterial leaf blight (*Erwinia stewartii*), common corn smut (*Ustilago maydis*), maize stripe virus and common maize rust (*Puccinia sorghi*).

These observations were obtained by comparing the general vigor and disease and insect susceptibility of MON 80100 and non-transgenic lines.

I. Yield and Quality Characteristics

The gene insertion has also been shown not to negatively affect yield. A comparison of a commercial hybrid with the same hybrid in which one parent was a BC3-derived MON 80100 line indicated no significant difference between the two hybrids in yield (Table V.3). The insect protected corn line MON 80100 that is the subject of this Determination for Non-regulated Status is still in development. Only those lines with commercially acceptable yield and quality characteristics will enter the marketplace.

Table V.3. Yield comparison of non-transgenic and MON 80100 version of the same hybrid.

	Bushels/ Acre[1]
Normal hybrid	186.0
MON 80100 BC3 hybrid	185.8

[1] means of 18 reps over 6 locations
lsd=8.8 bu/acre

J. Composition Analysis of Insect Protected Corn

In the U.S., corn is the largest crop in terms of the planted acreage, total production, and crop value with the majority of the national production produced in seven north central Corn Belt states (Olson and Sander, 1988; National Corn Growers Association, 1994). The low price and ready availability of corn has resulted in the development of large volume food and industrial uses of corn starch and converted corn products such as ethanol and high fructose corn syrup (Anderson and Watson, 1982; Watson, 1988).

Animal feeding, however, is by far the largest use of corn in the U.S. with more than half (50 to 60 percent) of annual production fed to swine, cattle, poultry, and dairy (U.S. Feed Grains Council, 1992; Perry, 1988; Watson, 1988). Starch, protein, and oil components of corn are the predominant fractions of animal and human nutrition interest. Although all are modifiable by traditional breeding, corn breeders have focused on agronomic properties including yield, maturity, and standability.

There is a relatively wide range of values for the nutritional components for corn in the literature (Watson, 1982). Protein, representing 8 - 14% of the kernel dry weight, is of low biological value as it does not supply the essential amino acids either in adequate quantities or proportions (Watson, 1982; Perry, 1988). Fat generally composes 3.9 -5.8% of dry weight (Watson, 1988; Perry, 1988) although higher values have been reported (Watson, 1982). Ash representing a relatively minor component (1.1 - 3.9% of dry weight), has nutritional value (Perry, 1988). Moisture content must generally be maintained below 15.5% for transport and storage (Watson, 1988) with literature values ranging from 7 - 23% (Watson, 1982). Carbohydrates are composed of multiple fractions including starch, the predominant constituent in corn grain, as well as fiber, sugars, water soluble polysaccharides, and phytate (Watson, 1982). Values reported vary depending upon the carbohydrate fraction reported and the method of calculation.

Compositional (proximate) analyses were performed on the corn seeds from insect protected corn line MON 80100 and the MON 80080 control line. Components measured were protein, fat, ash, fiber, and moisture. Carbohydrates were determined by calculation. Results from each of five sites of each line were statistically analyzed and the average values from these sites are shown in Table V.4. There were two statistically significant differences observed between line MON 80100 and the parental control line, MON 80080. The level of protein (13.1% vs. 12.0% for MON 80100 and MON 80080, respectively) was statistically significantly different but this minor difference falls well within the expected range of corn (Watson, 1982). The

value for carbohydrates was statistically significantly different. However, since the value for the percent protein was significantly different and the value for carbohydrate was determined by calculation (Table V.4, footnote g), this difference can be attributed to the difference in percent protein. Since the protein level falls within the expected range for corn, we conclude that there were no meaningful compositional differences between the insect protected corn line MON 80100 and the control line MON 80080.

Table V.4 Compositional analysis of corn seed from insect protected corn line MON 80100 and MON 80080 control seeds grown under field conditions.

	Values are Percentages [a]	
Component	MON 80100	MON 80080
Protein[b,c]	13.1 *	12.0
Fat[b,d]	4.0	4.0
Ash[b,e]	1.6	1.6
Fiber[b,f]	2.3	2.2
Carbohydrates[b,g]	81.3 *	82.2
Moisture[h]	14.8	14.6

* Values are significantly different at the 95% confidence level from the MON 80080 control line. SAS Institute Inc. 1990.

a Analyses of data from seed samples from 5 sites

b Dry weight basis

c Total Kjeldahl Nitrogen-Protein; Official Methods of Analysis of the AOAC, 15th Ed., 988.04C, 979.09.

d Fat, Ether Extraction; Official Methods of Analysis of the AOAC, 15th Ed., 960.39.

e Ash, Official Methods of Analysis of the AOAC, 15th Ed., 923.03.

f Fiber, Official Methods of Analysis of the AOAC, 15th Ed., 962.09.

g By calculation: % carbohydrate = 100% - (% protein + % moisture + % fat + % ash)

h Moisture, F.D., 100°C; Official Methods of Analysis of the AOAC, 15th Ed., 926.08, 925.09.

In addition to the proximate analysis shown in Table V.4, amino acids and fatty acids were determined for line MON 80100 and the control line MON 80080. These data will be provided to the FDA in support of the food/feed safety of insect protected corn. Based on these results, the insect protected corn line MON 80100 is comparable to the parental control line, MON 80080 and other commercial corn varieties.

K. Development of Pest and Resistance Management Strategies for Insect Protected Corn

Some organisms are resistant to single or multiple pesticides in use today. It has not been established whether this resistance is because the organism has adapted metabolically to be able to tolerate the effects of the pesticide, or that a small segment of the population was naturally resistant and dominate as the numbers of the susceptible members have been reduced. Regardless of how resistance is obtained, it is a potentially serious problem with some pests.

Some insect resistance to the *B.t.k.* insect control protein has been reported in the past five years. Examples of insects for which resistance has been reported are the Indianmeal moth (*Plodia interpunctella*), almond moth (*Caudra cautella*) and the diamondback moth (*Plutella xylostella*). There are also some examples of insecticides such as the organophosphates for which little resistance has been reported. In fact some of these chemical insecticides are able to control the same insects at the same dosages as when they were commercialized over 30 years ago.

It is currently not possible to accurately predict whether resistance will occur by an insect to an insecticide. Therefore, is important that every insecticide commercialized be used in a manner and as a part of an overall pest control program so as to maximize its usefulness. Monsanto is developing a pest control strategy aimed at reducing the probability of resistance becoming a problem. This strategy is included in Appendix IV of this Petition for Determination of Non-Regulated Status. This will be offered to growers choosing this corn seed. We believe by implementing these strategies, the development of resistance (if it occurs at all), can be managed to maximize the usefulness of this modified corn.

To achieve the benefits described above, it is important that insect protected corn be implemented and managed properly. In this respect, these plants are no different than any other crop protection product that has been used over the last century. It is clear from the knowledge gained over that time, that to successfully maximize the long-term use of insect protected corn, two interconnected management components are required. First, is the development of integrated pest management techniques that allow the farmer to optimize the utility of these plants for corn pest control. In essence, this is the development of a total insect management package that will be centered around insect protected corn. Second, to maximize the durability of this corn, is the development and implementation of strategies targeted to prevent the development of insect resistance to the insect control protein produced by these plants

Over this past year, consultations have been held with leading corn pest and resistance management researchers to develop a program to maximize the use and durability of insect protected corn. Laboratory and field studies designed in collaboration with these experts from academia and extension are in progress and are providing the data needed for developing this management program. These studies are examining the impact of insect protected corn on populations of beneficial and pest insects endemic to the crop, the impact on the use of conventional insecticides for controlling non-target pests, the establishment of the baseline susceptibility of our insect targets to the *B.t.k.* insect control protein, and the impact of mixtures of resistant and non-resistant plants on yield loss.

Monsanto scientists have worked for several years on laboratory and field studies of insect resistance, and with outside collaborators nearly every suggestion made for resistance management in insect protected corn is being examined. These strategies, developed in consultation with an expert advisory panel, take into account existing research and an understanding of corn production and agronomic practices. They include:

1) High dose expression of the *B.t.k.* insect control protein in corn to control caterpillars heterozygous for resistance alleles.

2) Refugia as hosts for sensitive insects provided through non-insect protected corn.

3) Monitoring of insect populations for susceptibility to the *B.t.k.* insect control protein.

4) Agronomic practices that minimize insect exposure to the *B.t.k.* insect control protein.

5) Development of novel lepidopteran control proteins with a distinct mode of action from the *B.t.k.* insect control protein.

Those pest and resistance management strategies best suited for use in corn production and with the potential for delaying or preventing the development of resistance will be recommended. A cooperative effort between growers, academia, extension, seed company partners and Monsanto will help ensure that the benefits of insect protected corn are fully realized and sustained.

Table V.5 Disease and insect susceptibility of insect protected corn line MON 80100 in comparison to non-modified corn plants.

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1992		
Kekaha, HI (92-209-02)	no	no
1993		
Kaunakakai, HI (92-209-03)	no	no
Isabela, PR (92-232-01)	no	no
Kihei, HI (92-265-01)	no	no
Jerseyville, IL (93-012-04)	no	no
Monmouth, IL (93-012-04)	no	no
Lockbourne, OH (93-012-04)	no	no
Aurora, IL (93-021-05)	no	no
Bloomington, IL (93-021-06)	no	no
Dekalb, IL (93-021-07)	no	no
Williamsburg, IA (93-021-08)	no	no
St. Joseph, IL (93-021-04)	no	no
Winterset, IA (93-021-03)	no	no
Johnston, IA (93-021-03)	no	no
York, NE (93-060-06)	no	no
Windfall, IN (93-060-06)	no	no
Salinas, PR (93-144-02N)	no	no
Kekaha, HI (93-146-02N)	no	no
Kekaha, HI (93-245-02N)	no	no
Loxley, AL (93-250-04N)	no	no
Kaunakakai, HI (93-258-04N)	no	no
Kaunakakai, HI (93-279-04N)	no	no
Kaunakakai, HI (93-308-02N)	no	no

Table V.5 Disease and insect susceptibility of insect protected corn line MON 80100 in comparison to non-modified corn plants (continued).

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1994		
Kaunakakai, HI (93-288-01N)	no	no
Isabela, PR (93-306-04N)	no	no
Kaunakakai, HI (93-316-04N)	no	no
Kunia, HI (93-354-06N)	no	no
Kaunakakai, HI (94-026-04N)	no	no
Santa Isabel, PR (94-026-04N)	no	no
Platteville, WI (94-033-04N)	no	no
Jerseyville, IL (94-060-03N)	no	no
Kihei, HI (94-074-11N)	no	no
Thomasboro, IL (94-074-11N)	no	no
Farmer City, IL (94-074-12N)	no	no
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

Table V.5 **Disease and insect susceptibility of insect protected corn line MON 80100 in comparison to non-modified corn plants (continued).**

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1994		
Wood River, NE (94-083-03N)	no	no
Slater, IA (94-083-03N)	no	no
Stanton, MN (94-083-04N)	no	no
O' Fallon, MO (94-105-014N)	no	no
Kaunakakai, HI (94-171-05N)	no	no
Santa Isabel, PR (94-171-05N)	no	no
Kaunakakai, HI (94-279-03N)	no	no
Santa Isabel, PR (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
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
Princeton, IN (94-024-10N)	no	no
Miami, MO (94-024-12N)	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

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Part VI. Statement of Grounds Unfavorable

We know of no unfavorable grounds associated with insect protected corn line MON 80100, developed using the plasmid vectors, PV-ZMBK07 and PV-ZMGT10. Therefore, on the basis of the substantial potential benefits to the grower, the environment, and the consumer, Monsanto Company requests that this corn line no longer be regulated under 7 CFR part 340.6.

APPENDIX I

AGRONOMIC BENEFITS OF CORN GENETICALLY MODIFIED TO RESIST EUROPEAN CORN BORER AND OTHER LEPIDOPTERAN PESTS

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Agronomic Benefits of Corn Genetically Modified to Resist European Corn Borer and Other Lepidopteran Pests

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Executive Summary

Insect protected corn will provide excellent control of an insect (European corn borer) that causes significant decreases in corn yields every year in North America. Results from field experiments with Monsanto's genetically modified corn expressing delta-endotoxin proteins of *Bacillus thuringiensis* var. *kurstaki* (insect protected corn) revealed a high level of efficacy against European corn borers, *Ostrinia nubilalis* (Hübner), in 1994. The results from field trials in Kansas indicated that Monsanto's transgenic corn also is efficacious against southwestern corn borers, *Diatraea grandiosella* Dyar. Southwestern corn borers occur in areas of the western Corn Belt where this pest causes yield reductions. Control of other lepidopteran pests with insect protected corn may also be possible, and is currently being investigated for armyworms, corn earworms, fall armyworms, and stalk borers in corn.

Currently, corn growers in the eastern Corn Belt treat relatively few acres annually with insecticides to control European corn borers. Corn growers in Colorado, Kansas, and Nebraska treat comparatively more acres to control corn borers. However, yield losses attributable to corn borer damage are appreciable throughout its range. One study in Illinois (Briggs & Guse 1986) revealed that approximately 10 percent of the corn acres in that state experience a 9- to 15-percent yield loss annually, attributable solely to the damage caused by the second generation of corn borers. [At least two generations of this pest occur annually throughout most of the Corn Belt.] Results from several studies suggest that corn borers cause an estimated 5 to 7.5 percent yield loss annually (first and second generations combined) (Bergman et al. 1985a-f; Bode and Calvin 1990). These data induce entomologists throughout the United States to consider the European corn borer to be the most under-scouted and under-treated insect that attacks corn. Because European corn borers cause primarily physiological reductions in yield, corn growers are not aware of the significance of their feeding injury during years when infestations are moderate. In addition, efficacy of insecticides applied for control of corn borers is often less than acceptable, particularly for the second generation. Both timing of insecticide applications and placement of the insecticide where corn borer larvae are feeding are difficult. Corn growers frequently are dissatisfied with the level of control of corn borers provided by both chemical and microbial insecticides.

The only other management tactic currently utilized for management of European corn borers is planting of resistant or tolerant corn hybrids. Entomologists and corn breeders have attempted for many years to develop hybrids resistant to European corn borers. However, although some hybrids are resistant to first-generation corn borers, none are resistant to second-generation borers. Some hybrids also have the ability to tolerate an infestation of corn borers. Nevertheless, planting of corn hybrids specifically because they are resistant to European corn borers is not widespread, and tolerant hybrids often do not yield as well when infestations of corn borers are heavy.

Insect protected corn promises to be a profound breakthrough in corn insect management. Corn growers who plant insect protected corn will experience yield protection during years when infestations of European corn borers are moderate to large. The potential for substantial reduction or virtual elimination of insecticide use for corn borer control is real. Additionally, the selective activity of the *Btk* endotoxins will not disrupt populations of either beneficial insects or nontarget animals (e.g., birds, fish). Applications of conventional chemical insecticides often affect nontarget species.

The development of insect protected corn may become a foundation for corn insect management throughout the United States. Reduced insecticide use and improved yields are the likely outcomes of implementation of this technology. If growing insect protected corn effectively eliminates all insecticide applications for European corn borers, corn growers would save a conservative $50 million annually. [This figure was derived from an estimate of 5 percent of the acres of corn treated with insecticides for corn borer control and $15 per acre control costs (insecticide + application costs).] The yield protection benefits gained from controlling corn borer infestations are between 1 and 1.5 billion dollars. [This figure was derived from annual estimates of 70 million acres of corn, an average yield of 120 bushels per acre, an average corn price of $2.35 per bushel, and an estimated 5 to 7.5 percent yield loss attributed to corn borer damage.]

The development of insect protected corn will have a major impact on corn pest management. The reduction in the use of aerially applied insecticides will preserve many beneficial insects, and the integration of insect protected corn with other forms of resistance or tolerance will provide solid footing for the development of nonchemical technologies for other major insect pests.

Introduction

Corn, *Zea mays* (L.), is the major feed grain grown in the United States, leading all other crops in both value and volume of production. After rice and wheat, corn ranks as the third most important cereal food crop of the world. It is grown annually on 65 to 70 million acres, mostly in the north central states. Approximately 75 percent of the corn is grown in only 10 states. In 1993, growers in Iowa harvested 11 million acres of corn; Illinois, 10 million acres; Nebraska, 7.55 million acres; Indiana, 5.4 million acres; Minnesota, 4.6 million acres; Ohio, 3.28 million acres; South Dakota, 2.55 million acres; Wisconsin, 2.35 million acres; Michigan, 2.15 million acres; and Missouri, 1.85 million acres. The United States annually produces 7.5 billion bushels of corn at a value of 16.5 billion dollars (National Corn Growers Association 1994).

Insects are among several pests that can reduce corn yields and hamper corn production. Dicke (1977) listed 79 insects that may infest corn in the Western Hemisphere. Gray & Luckmann (1994) sorted 34 different insects that attack corn into three categories: (1) major and consistent pests, (2) major but sporadic pests, and (3) pests of moderate to minor importance. Insects considered to be major and consistent pests are the western corn rootworm [*Diabrotica virgifera virgifera* LeConte], the northern corn rootworm [*Diabrotica barberi* Smith & Lawrence], the corn earworm [*Helicoverpa zea* (Boddie)], the fall armyworm [*Spodoptera frugiperda* (J.E. Smith)], and the European corn borer [*Ostrinia nubilalis* (Hübner)].

Corn is protected from insect pests mainly by cultural practices, insecticides, insect-resistant and insect-tolerant corn varieties developed from traditional breeding, and the effects of predators, parasitoids, and pathogens (Gray & Luckmann 1994). In general, two distinct groups of insect pests are managed by growers--insects that attack corn plants below ground and insects that attack corn plants above ground. The below-ground pests, including corn rootworms, cutworms, wireworms, and white grubs, are managed primarily through crop rotation and the use of soil insecticides applied before planting, at planting, or shortly after corn emergence. The above-ground pests, including the European corn borer, southwestern corn borer, corn earworm, and fall armyworm, are managed primarily through scouting programs and application of an insecticide if the pest density exceeds an established economic threshold.

Because of the type of injury they cause and their sometimes unpredictable occurrence, below-ground insect pests have long been considered as the most important insect pests of corn. Corn growers spend a great deal of money on soil insecticides to prevent damage that might be caused by these pests. In 1978, growers in Illinois treated 65 percent of their corn acres with soil insecticides (Owen 1980). Although insecticide use on corn has diminished since the late 1970s, farmers in Illinois still treated approximately 33 percent

of their corn acres in 1990 (Pike et al. 1991), representing an estimated expenditure of 39.6 million dollars. However, recent evidence suggests that many acres of corn in Illinois are being treated unnecessarily (Gray et al. 1993), i.e., soil insecticides are being applied prophylactically without knowledge of insect densities within fields.

Despite the large expenditure for soil insecticides to control below-ground pests of corn, many entomologists consider the European corn borer to be the most economically damaging insect pest of corn in North America. Burkhardt (1978) estimated that the cost of corn borer damage exceeds 210 million dollars annually. However, for reasons that will be explained in this document, the impact of European corn borer injury on corn yields exceeds most farmers' recognition of economic losses, i.e., corn borers cause more economic damage than farmers realize. In 1991, the year in which the most recent serious outbreak of European corn borers occurred, growers treated only approximately 5.5 percent of the corn acres in the United States (Maritz Marketing Research Inc. 1994). However, in Illinois alone, Stout (1991) estimated that corn borers cost Illinois producers approximately $36 per acre, based upon an average yield of 110 bushels per acre, a market price of $2.45 per bushel, a preharvest average of 3.3 borers per plant, and a 4 percent physiological yield loss per borer.

The primary objective of this document is to describe the agronomic benefits that will likely accrue to corn genetically modified to resist European corn borers. Potential agronomic benefits associated with control of other lepidopterans (caterpillars) will be discussed briefly. The objectives will be addressed through discussions of:

(1) the impact of European corn borer injury on corn production in the United States
(2) current management options for European corn borers
(3) benefits of corn genetically modified to resist European corn borers and other lepidopterans

Impact of European Corn Borer Injury on Corn in North America

A Brief Early History of European Corn Borers in North America

The European corn borer was first identified in the United States in 1917 in a sweet corn field near Boston, Massachusetts, although it was probably introduced as early as 1910 on broom corn imported from Italy or Hungary (Caffery & Worthley 1927). In Europe, in the late 1800s and early 1900s, borer densities averaged 15 to 20 larvae per plant, with maximum numbers as high as 40 to 60 per plant (Parks 1926).

During the early 1920s, corn borers found in the north central states had only one generation each year. However, the "New England strain" discovered in 1917 had two generations per year. This strain spread gradually westward and southward between 1927 and 1936. The two "strains," now referred to as ecotypes (Showers et al. 1975), probably represent two separate introductions into North America (Brindley & Dicke 1963). Currently, corn borers throughout most of the central Corn Belt have two generations per year, those in the northern United States and Canada have only one generation per year, and those in the southern United States have three to four generations per year (Showers et al. 1989).

After 1936, the spread of European corn borers was rapid. It was first found in Illinois in Lake County (northeast) in 1939, in the prairie provinces of Canada in 1949, and as far west as the Rocky Mountains and as far south as Alabama, Georgia, and Mississippi by 1950 (Briggs & Guse 1986). Its range now occupies 40 states and 8 Canadian provinces (Palmer et al. 1985; Showers et al. 1989).

Seasonal History of European Corn Borers

Thorough discussions of the biology and seasonal history of corn borers are offered by Showers et al. (1989) and Hudon et al. (1989). Following is a brief account of the seasonal history of the corn borer and how its feeding affects corn plants.

The European corn borer overwinters as a mature larva (fifth instar) within the stalk, stubble, or ear of corn. Diapause is induced by a combination of photoperiod and thermoperiod. Consequently, the corn borer is univoltine (one generation per season) in northern areas where the growing season is shorter, is bivoltine (two generations) in the central Corn Belt, and is multivoltine (multiple generations) in the southern areas of its range. In the spring, the larva transforms into a pupa, and the moth emerges shortly thereafter. Shortly after emergence, the females mate and seek out hosts on which to lay their eggs.

In areas where there are two generations, corn borer moths laying eggs for the first generation typically seek the earliest planted corn (the tallest corn) on which to deposit their eggs. The females lay eggs in masses on the undersides of the leaves near the midrib. The eggs hatch in a few days, and the young instars move immediately into the whorl leaves. Young first-generation larvae feed on leaf tissue within the whorls. After their third molt, the larvae bore into the leaf midrib or into the stalk to complete their development. Their tunneling activity within the stalk interferes with the transfer of water and nutrients within the plant. Consequently, their feeding reduces yield potential. In addition, the holes in the stalks provide an avenue for the entrance of stalk rotting organisms.

After first generation corn borer larvae complete their feeding and development, they pupate within the stalk, transform into moths, and emerge to mate and begin laying eggs for the second generation. The oviposition period during this second flight of moths can extend for several weeks. Moths laying eggs for the second generation are usually attracted to pollinating fields if they are available. Again they lay their eggs on the undersides of leaves near the midrib, usually within the ear zone (the three leaves above the ear, the ear leaf, the three leaves below the ear, and the associated stalk region). Upon hatching, the larvae feed on pollen that accumulates in the leaf axils and on sheath and collar tissues. Third instars begin tunneling into the midribs, ears, ear shanks, and stalks. Their feeding can cause a physiological reduction in yield and can weaken both the stalks and shanks, resulting in broken stalks and dropped ears. Badly damaged fields are sometimes difficult to harvest. In areas where two generations occur, mature larvae enter diapause when conditions are appropriate.

Late in the summer, particularly in the mid-southern United States, another flight of moths lays eggs for a third generation. In the southern United States, a fourth generation may occur.

Levels of Infestation of European Corn Borers

As previously mentioned, the first European corn borers in Illinois were found in Lake County in 1939. The first severe infestation of corn borers in Illinois was found in Kankakee County in 1941. By 1942, corn borers were found as far west in Illinois as the Mississippi River, and by the mid-1940s, corn borers could be found in every county in the state (Briggs & Guse 1986).

Because of its devastating effect on corn production in the United States, the European corn borer became the focus of national surveys supported by the United States Department of Agriculture. Surveys of fall populations of corn borers were undertaken annually in affected states. Unfortunately, as funding for this program began to diminish, many states discontinued their surveys, consequently making the national impact of corn borers on corn yield difficult to assess. However, entomologists and their cooperators in Illinois have conducted an annual fall survey from 1943 to the present and probably have the most extensive records of densities of European corn borers in the United States. Between 30 and 60 counties within the state have been surveyed every year. Within each county, surveyors randomly select 10 fields distributed across the county. Within each field, the percentage of plants infested and the average number of corn borer larvae per infested plant are estimated. Results from these annual surveys provide an example of the fluctuations of corn borer densities for more than 50 years in a major corn producing state.

The statewide average numbers of corn borer larvae per plant in Illinois from 1943 through 1994 are shown in Figure 1. The numbers reveal that the largest densities of European corn borers in Illinois, with greater than an average of 2 corn borers per plant, occurred in 1949, 1955, 1968, 1978, 1986, 1989, and 1991. The two most recent outbreaks in 1989 and 1991 were the second and third largest densities, respectively, since 1949. Metcalf & Metcalf (1993) estimated that the corn borer outbreak in 1949 caused a loss of 313 million bushels of corn and total damage in excess of 349 million dollars. Using figures mentioned previously, Stout (1991) estimated economic losses caused by corn borers in Illinois in 1991 to be 360 million dollars. Unfortunately, because data regarding infestations of first generation corn borers are lacking, the economic losses estimated for 1991 represent losses attributable only to the second generation of borers for which data were gathered.

Data from the annual fall surveys in Illinois taken from 1986 through 1994 (Table 1), the most readily accessible data, show the average percentage of stalks infested by European corn borers. Multiplying the percentage of stalks infested by the appropriate average number of corn borer larvae per infested stalk for the same year provides an estimate of the average number of corn borers per plant. For the most recent severe outbreak of corn borers in Illinois (1991), 91.4 percent infestation times 3.3 larvae per infested stalk provides an estimated 3.02 larvae per plant. If one uses the same figures used by Stout (1991), i.e., 110 bushels per acre, $2.45 per bushel, and 4 percent yield loss per borer per plant, even the more conservative estimate of 32.5 million dollars loss caused by corn borers in Illinois is impressive.

Although some research has indicated that damage caused by second generation corn borers is more significant (Foott & Timmins 1981), many researchers (e.g., Lynch et al. 1980b) believe that in areas where two generation corn borers are common, the first generation causes more damage than the second generation. Consequently, yield loss estimates based solely on fall surveys of second generation borers represent only part of the economic impact of corn borers. The nationwide impact of damage caused first generation corn borers has not been studied sufficiently.

The levels of infestation of European corn borers from elsewhere in North America are less well documented. From surveys conducted in Iowa, Foster & Tollefson (1986) determined that only 8 percent of the fields surveyed had an infestation level of corn borers that exceeded 35 percent, a commonly used economic threshold in the mid-1980s. However, the surveys were conducted during years when corn borer densities were not exceedingly large. Densities of European corn borer larvae from multiple states and multiple years were examined by members of the USDA NC-180 (now NC-205) regional research project regarding stalk boring insects. These data revealed a mean density of 1.37 larvae per plant.

Economic Impact of European Corn Borer Injury on Corn Yield

A great deal of research has been devoted to determining the effect that European corn borers have on the yield of corn. A complete listing of the published literature would be lengthy, so only the most recently published papers are offered: Berry et al. (1978), Bode & Calvin (1990), Boivin et al. (1988), Briggs & Guse (1986), Foott & Timmins (1981), Hudon et al. (1992), Lynch (1980), Sorenson et al. (1993), and Umeozor et al. (1985). The information from these studies was gathered from widely disparate locations, from univoltine populations in Canada, bivoltine populations in the central Corn Belt, and multivoltine populations in North Carolina. Consequently, the combined findings represent the overall economic impact of European corn borers on corn yields throughout most of its range.

The study by Lynch (1980) is the classic study from which many economic inferences have been made and upon which many state's economic thresholds are based. Lynch (1980) showed that the effect of corn borers' feeding on corn plants changed with the stage of growth of corn at the time of attack; subsequent research has supported his findings. His percentage yield loss figures attributed to each corn borer per plant are utilized in decision-making worksheets throughout the Corn Belt [see Figure 2, from Gray & Steffey (1995), as an example]. Truncating Lynch's (1980) data, Gray & Steffey (1995) suggest the following yield loss estimates: 5 percent per borer per plant for corn in the early whorl stage; 4 percent for corn in the late whorl stage; 6 percent for corn in the pretassel stage; 4 percent for corn that is shedding pollen; and 3 percent for corn in which kernels have been initiated.

In a series of Pesticide Impact Assessment Program reports, Bergman et al. (1985a-f) reported that the average percentage yield loss attributed to corn borers in six different regions of the United States ranged from 0.6 to 3.9 percent when current controls were used and 2.6 to 7 percent when no insecticides were used. Percentage yield losses attributed to corn borers in these six regions of the United States are listed in Table 2.

More recently published research with more modern corn hybrids grown in Pennsylvania (Bode & Calvin 1990) showed that the yield loss figures determined by Lynch (1980) are still valid. Bode & Calvin (1990) estimated that on average a first generation larva reduces corn yield by 5.5 percent and that a second generation larva reduces corn yield by 2.8 percent. The combined annual losses caused by both generations of corn borers would be 8.3 percent. These data and the information generated in the Pesticide Impact Assessment Program reports suggest an estimate of about 5 to 7.5 percent yield loss caused by both generations of corn borers. If one assumes that corn is grown on 70 million acres, the average yield is 120 bushels per acre, and the average price received for corn is $2.35 per bushel, the annual loss attributable to corn borers in the United States is 1 to 1.5 billion dollars.

Current Management Options for European Corn Borers

Throughout the European corn borer's range in North America, corn growers rely upon natural suppression of corn borer densities and several applied tactics to integrate management of this most important insect pest. Unfortunately, none of the methods of control used over time (cultural, biological, chemical, breeding for crop resistance) has alone, or when integrated, provided an acceptable sustained level of borer control (Hudon et al. 1989). Nevertheless, currently available options for management of corn borers have received considerable attention. A detailed discussion of management options in the United States appears in Showers et al. (1989). Examples of Illinois' suggestions for management of European corn borers are published in Gray & Steffey (1995) and Weinzierl et al. (1995).

Cultural Tactics

Although fall moldboard plowing, or clean plowing, was originally recommended widely as a control measure for European corn borers, current concerns about soil conservation make clean plowing an inappropriate management tactic. In fact, many researchers have found that the effect of tillage on corn borer densities is not consistent over time. Where reduced tillage leads to delayed planting or slower germination due to cooler soil temperatures, corn may be less susceptible to attack by first generation borers and more susceptible to attack by second generation borers.

Although reduced tillage favors greater survival of corn borers in crop residue, effects in specific fields are minor because moths disperse from emergence sites to lay eggs in suitable fields throughout the local area. Therefore, the impacts of tillage are not confined to a single field, regardless of the tillage practice employed. For the same reason, stalk shredding in the fall, another tactic that has gained some recent interest, will not necessarily provide control of corn borers within a given field. Stalk shredding would have to be adopted over a large area if the ultimate goal were to reduce corn borer densities.

Time of planting probably has a greater impact on corn borer densities than any other cultural practice. Early planted corn is more attractive to moths laying eggs for the first generation because the moths prefer to deposit their eggs on whorl stage corn with an extended leaf height of at least 18 inches. Conversely, late planted corn is more attractive to moths laying eggs for the second generation. Showers et al. (1989) suggested that planting long season corn with heavy fertilization probably has contributed to larger borer densities, particularly in areas where corn is irrigated. Corn hybrids planted in those areas are usually long season and are planted very early, making the corn attractive to both generations of the corn borer.

Intercropping, the practice of growing two or more crops simultaneously in one field, has received some attention related to corn borer management. Some ecologists theorize that intercropping increases the diversity of plant species, thereby reducing the impact of pest species. However, numerous studies of agricultural systems with increased diversity have not revealed consistent findings. Nevertheless, research conducted in Ontario, Canada (Lambert et al. 1987) showed reductions in infestations of European corn borers in corn where red clover was interseeded within 10 days of corn planting. Corn yields were not reduced in the mixed-crop fields.

Suppression of European Corn Borer Densities by Predators, Parasitoids, and Pathogens

Although predators, parasitoids, and pathogens affect densities of European corn borers, their combined suppressive effects usually are not long term. Hudon & LeRoux (1986) found that indigenous natural enemies of corn borers were scarce throughout the period of their study. Although the annual incidence of mortality from all factors (including weather) varied from 96.8 to 99.4 percent in their study, an overall survival rate of only 1.3 percent was sufficient to sustain economic levels of infestation.

The most common predators of European corn borers, primarily predators of the eggs, include coccinellids (especially *Coleomegilla maculata*), chrysopids, nabids, syrphids, pentatomids, and mirids. Birds may also play a role in predation of corn borers. However, the impact of predators on densities of corn borers during most years is relatively minor (Andow 1992; Hudon & LeRoux 1986; Jarvis & Guthrie 1987).

The most common parasitoids of European corn borers, both introduced from Europe, are *Eriborus terebrans* (Hymenoptera: Ichneumonidae) and *Macrocentrus grandii* (Hymenoptera: Braconidae). *Lydella thompsoni* (Diptera: Tachinidae), another introduced parasitoid, is no longer found in much of the United States. The impacts that parasitoids have on densities of corn borers have been relatively minor in most published ecological studies (Landis & Haas 1992; Lewis 1982; Mason et al. 1994; Onstad et al. 1991). However, Siegel et al. (1987) found that *M. grandii* was an important first generation mortality factor. Landis & Haas (1992) indicated that a more thorough understanding of the role of landscape structure and potential manipulation of cultural tactics could improve the rate of parasitism of European corn borers.

Attempts to control European corn borers by inundative releases of two parasitoids, *Trichogramma minutum* and *T. nubilale* (Hymenoptera: Trichogrammatidae), have shown some promise in Europe. Kanour &

Burbutis (1984) recommended specific release rates for these parasitoids, but experimentation with this practice in North America has only just begun (Prokrym et al. 1992). In general, research on the use of insect parasitoids and predators as biological control agents has had little impact on borer management in North America (Hudon et al. 1989).

The most important natural control agent of European corn borers in North America is the microsporidian *Nosema pyrausta* (Microspora: Nosematidae). Several researchers have studied the impact of this disease on corn borer populations and have witnessed significant levels of infection and subsequent reductions in corn borer numbers (Andreadis 1984, 1987; Hill & Gary 1979; Lewis & Cossentine 1986; Lewis & Lynch 1978; Siegel et al. 1987). The overall impact of *Nosema pyrausta* on corn borers is a reduction in egg hatch, a slowing down of larval development, and a reduction in fecundity and longevity of adults. Some researchers have also studied the effects of foliar applications of *Nosema pyrausta* as an applied pest management tactic (Lewis & Lynch 1978; Lublinkhof et al. 1979). Unfortunately, *Nosema pyrausta* has occasionally had a negative impact on the parasitoid *Macrocentrus grandii* (Cossentine & Lewis 1987; Siegel et al. 1987; Orr et al. 1994).

Beauveria bassiana (Deuteromycotina: Hyphomycetes) is an entomopathogenic fungus that is often found infecting corn borer larvae late in the season (Bing & Lewis 1993). Its suppressive effects on corn borer densities have been relatively minor. However, its use as an applied borer management tactic may hold some promise (Lewis & Cossentine 1986).

Host Plant Resistance

Since the recognition of the economic impact that European corn borers can have on corn production, entomologists have worked with plant breeders in numerous attempts to develop corn hybrids that are resistant to corn borers. Host plant resistance studies have been carried out in North America for at least 60 years. These studies have led to the development of a number of corn genotypes with a satisfactory degree of resistance to leaf feeding by first generation borers and univoltine borers (Hudon & Chiang 1985). However, developing genotypes resistant to second generation borers has remained rather elusive (see Hudon et al. 1989 for further references).

Screening trials conducted by seed companies since the 1960s have selected hybrids less susceptible to corn borer infestation, stalk breakage, ear drop, and yield reduction. However, resistance factors have not always been identified. In some varieties, high levels of DIMBOA and other factors in young corn plants cause mortality of first-generation corn borers (Barry et al. 1994). Other unidentified factors cause reduced tunneling by second generation borers.

Barry & Darrah (1991) reported on their evaluations (1986 through 1989) of 100 commercial corn hybrids for resistance to first and second generation corn borers in Missouri. They found that about 90 percent of the hybrids examined had some resistance to whorl leaf feeding, and approximately 75 percent offered some resistance to sheath and collar feeding. In an attempt to inform growers that different hybrids have different levels of susceptibility to corn borers, Illinois entomologists published (Steffey et al. 1992) the names of 17 of the hybrids that showed high levels of resistance to both generations. However, because lists of available corn hybrids change rapidly, such a published list quickly becomes outdated.

Showers et al. (1989) reported that beginning in the mid-1970s, the number of acres planted with corn hybrids resistant to leaf feeding had decreased substantially. They attributed this decrease to the extensive use of an inbred line in corn breeding programs that has excellent yield potential but is highly susceptible to corn borer damage. Corn growers typically select a corn hybrid based primarily on its yield potential, rather than on its resistance to corn borers. However, data from Bergman et al. (1985a, 1985d, 1985e, 1985f) indicated that corn borers were managed by varietal selection on the following percentages of corn acres in given states: 10 percent in Illinois; 15 percent in Iowa; 20 percent in Delaware and Maryland; 44 percent in North Carolina; 15 percent in Nebraska.

Insecticides and Insecticide Use

Because the combination of weather, natural enemies, and insect pathogens do not always suppress densities of European corn borers below economic levels, corn growers have to resort to application of insecticides during outbreaks of the pest. Insecticides applied with high-clearance sprayers, with aircraft, or through irrigation systems can offer effective control of economic levels of corn borers (Raemisch & Walgenbach 1983; Witkowski 1980; Witkowski et al. 1986). Several carbamate, organophosphorus, and pyrethroid insecticides are currently labeled for control of European corn borers in corn.

Pesticide use surveys have verified that a relatively low percentage of corn acres is treated for control of corn borers, even during years when their densities are quite large. Bergman et al. (1985a) reported that approximately 7 percent of the corn acres in Illinois, Indiana, Iowa, Missouri, and Ohio were treated for control of corn borers. Approximately 20 percent of the corn acres in Kentucky and Tennessee were treated for control of corn borers (Bergman et al. 1985b); 2 percent in the Lake states (Michigan, Minnesota, Wisconsin) (Bergman et al. 1985c); 5 percent in the northeastern states (Delaware, Maryland, New Jersey, New York, Pennsylvania, Virginia) (Bergman et al. 1985d); and 5 percent in the southeastern states (Alabama, Georgia, North

Carolina, South Carolina) (Bergman et al. 1985f). Relatively more acres (13 percent) were treated for corn borer control in the northern plains states (Colorado, Kansas, Nebraska, North Dakota, and South Dakota) (Bergman et al. 1985e). More recent pesticide use surveys reveal the same trend. Pike et al. (1991) reported that in 1990, only 361,900 acres of corn were treated for control of first generation corn borers in Illinois; only 22,700 acres were treated for control of second generation borers. Maritz Marketing Research Inc. (1994) reported that the following number (and percentage) of acres of corn throughout the United States were treated for control of corn borers in the respective years: 2.5 million acres (3.6 percent) in 1989; 3.3 million acres (4.5 percent) in 1990; 3.8 million acres (5.1 percent) in 1991; 2.6 million acres (3.4 percent) in 1992; and 1.9 million acres (2.7 percent) in 1993.

Pesticide use data from selected individual states (Maritz Marketing Research Inc. 1992, 1993) show that a higher percentage of acres of corn are treated with insecticides for control of corn borers in the western states than in the central Corn Belt states, in agreement with the surveys published by Bergman (1985a, 1985e). In 1992, insecticides were applied for control of corn borers on 1.3 percent of corn acres in Illinois, 1.7 percent of corn acres in Iowa, 3.6 percent of corn acres in Kansas, 2 percent of corn acres in Minnesota, and 7.4 percent of corn acres in Nebraska. In 1993, insecticides were applied for control of corn borers on 3.5 percent of corn acres in Indiana, 1.4 percent of corn acres in Iowa, 20 percent of corn acres in Kansas, <1 percent of corn acres in Minnesota, and 4.9 percent of corn acres in Nebraska.

Data from Monsanto's survey (unpublished) of 312 crop consultants indicated that during years when corn borer infestations are high, 56 percent of the corn acres should be treated; during years when the infestations are medium, 31 percent of the acres should be treated; and during years when infestations are low, 10 percent of the acres should be treated. These estimated acreages reflect the consultants estimates of typical losses that would occur on those acres that should be treated: 18 percent when infestations are high, 10 percent when infestations are medium, and 5 percent when infestations are low. These estimates of the corn acres that should be treated for corn borer control are considerably higher than the actual acreage of corn that is treated. However, these differences probably reflect the intensity of scouting efforts exercised by professional consultants.

One of the primary reasons why more acres are not treated with insecticides to control economically damaging densities of European corn borers is a lack of scouting efforts. In their series of Pesticide Impact Assessment Program reports, Bergman et al. (1985a-f) revealed that in many areas of the United States the percentage of acres scouted specifically for corn borers was usually relatively small: 25 percent in Illinois; 2 percent in Iowa; 1 percent each in Michigan, Minnesota, Ohio, and Wisconsin; 4 percent in

Kentucky; 30 to 40 percent each in Delaware and Maryland; 7 percent in North Carolina; 44 percent in Colorado; 29 percent in Kansas; and 7 percent in Nebraska. In general, more acres were scouted in the western states. In Illinois, the percentage of acres of corn scouted for all pests increased from 44 percent in 1982 to 62 percent in 1990. However, the percentage of acres scouted by professional consultants has changed very little from 3 percent in 1982 to 7 percent in 1990 (Pike et al. 1991).

Since the discovery of the insecticidal properties of *Bacillus thuringiensis*, entomologists have conducted numerous studies to determine the efficacy of various formulations of microbial insecticides containing *B. thuringiensis*. *Bacillus thuringiensis* var. *kurstaki* has been tested against European corn borers for more than 30 years (Hudon 1963; Hudon & Martel 1977; Lynch et al. 1977; Lynch et al. 1980a; McWhorter et al. 1972). Although nearly all studies have revealed that appropriate timing of applications of *B. thuringiensis* will provide efficacious control of young corn borer larvae, growers' and applicators' use of microbial insecticides containing *B. thuringiensis* to control corn borers remains relatively low. Inappropriate timing of application of *B. thuringiensis* is most often responsible for failure to control first generation corn borers; in appropriate timing of application and a lack of residual activity are often blamed for failures to control second generation corn borers. Recent research by McGuire et al. (1994) indicates that an experimental starch encapsulation process might extend the residual efficacy of formulated *B. thuringiensis*.

Despite the fact that research reveals reasonable levels of efficacy of insecticides against European corn borers, many acres of corn infested with economic levels of corn borers are never treated. As stated previously, many corn growers do not have a complete understanding of the overall economic impact of corn borers on corn yield. In addition, because of a lack of scouting efforts and poor timing of insecticide applications, controlling corn borers with insecticides is not always as effective in growers' fields as it has been in research trials. The occurrence of more than one ecotype and/or variability in flight patterns of corn borer moths in a given area further exacerbate timing of insecticide applications (Calvin & Song 1994; Eckenrode et al. 1983; Sorenson et al. 1992).

Benefits of Corn Genetically Modified to Resist European Corn Borers and Other Lepidopterans

The advent of genetic engineering technology to place the delta-endotoxin gene from *Bacillus thuringiensis* into crop plants has elicited a number of discussions regarding the potential, benefits, and risks of the technology in agriculture (Llewellyn et al. 1994; Meeusen & Warren 1989; Pimentel et al.

1989; Tiedje et al. 1989; Vaeck et al. 1987). Several crops have already been genetically modified to resist several pests, and the efficacy of these transgenic crops against several target pests have been published in the scientific literature: cotton (Benedict et al. 1992; Benedict et al. 1993; Jenkins et al. 1993; Perlak et al. 1990; Umbeck et al. 1987; Wilson et al. 1992; Wilson et al. 1994), poplar (Robison et al. 1994), potato (Cheng et al. 1992; Ebora et al. 1994; Perlak et al. 1993), and tobacco (Barton et al. 1987; Hoffman et al. 1992; Warren et al. 1992), and tomato (Delannay et al. 1989; Fischhoff et al. 1987).

Genetically modified corn expressing the delta-endotoxin proteins of *Bacillus thuringiensis* var. *kurstaki* (insect protected corn; IP-corn) is a relatively new technology developed for management of insects. Koziel et al. (1993) reported that corn plants expressing high levels of the insecticidal protein exhibited excellent control of repeated heavy infestations of European corn borers. Ebora et al. (1994) demonstrated that transgenic potatoes expressing the delta-endotoxin of *Bacillus thuringiensis* var. *kurstaki* also reduced survival of corn borer larvae.

Initial field studies with IP-corn show great promise for managing one of the most serious insect pests of corn in North America. Barrido & Steffey (1995) reported on a study in Illinois in which insect protected corn effectively reduced the amount of damage caused by European corn borers. In their trial, non-IP-corn generally had more leaf injury, broken tops, entry holes, and inches of stalk injury than IP-corn. The only treatment containing non-IP-corn that consistently produced results similar to those in IP-corn plots was the treatment that was sprayed weekly with a pyrethroid, Pounce® 3.2EC, to eliminate virtually all corn borers and other insect pests. In that same trial, two non-IP-corn plots that were treated weekly with Pounce 3.2EC to eliminate first generation corn borers had approximately the same yield as all five of the IP-corn treatments. A total of only 5 bushels per acre separated the top seven treatments. All IP-corn treatments produced higher yields than non-IP-corn treated with standard chemical (Pounce® 1.5G) or *Btk* (DiPel® 10G) insecticides or not treated at all--7.7 bushels per acre between the lowest yielding IP-corn treatment and the highest yielding non-IP-corn treated with a conventional insecticide; 19.3 bushels per acre between the lowest yielding IP-corn treatment and nontreated non-IP-corn. Similar successes regarding yield protection with IP-corn against European corn borers have been reported by Rice & Pilcher (1994) and Showers (1994).

Advantages of Insect Protected Corn

The use of IP-corn for management of European corn borers offers several advantages over many of the currently employed management tactics and should overcome the limitations associated with scouting for corn borers and timing insecticide applications. Growing IP-corn should reduce or eliminate the need for insecticides applied for control of corn borers. The associated costs of insecticide application should also be reduced or eliminated. Reductions in the potential for contamination of ground and surface waters and the potential for negative impacts against nontarget organisms should ensue. Control of corn borers with IP-corn is so effective that tissues within the corn plants that previously were hard to protect should be protected from attack by the borers. The need to scout for corn borers, a difficult task, especially for the second generation, will be reduced. Growers will also become less dependent on favorable weather conditions, as they currently are, if they rely on insecticide applications.

Reduction or elimination of insecticide use

Current reliance on insecticides to control economically damaging levels and outbreaks of European corn borer larvae can be replaced, or at least significantly reduced, with the use of IP-corn. In addition, the availability of new insecticides that could be registered for control of corn borers is declining. Also, the United States Environmental Protection Agency currently is studying the benefits and risks of all granular insecticides (Edwards 1991), the formulation of insecticides used by many applicators for control of corn borer larvae. The loss of these insecticides would have a significant impact on the growers ability to control corn borer larvae effectively, unless IP-corn were available.

Over the past five years, an average of 2.82 million acres of corn in the United States have been treated with insecticides to control first and second generation European corn borers (Maritz Marketing Research Inc. 1994). If growing IP-corn completely replaces the use of these conventional insecticides, the reduction in the amount of insecticides introduced into the environment would be substantial. If one assumes an average cost of applying an insecticide to be $15 per acre, the savings from not applying insecticides would be more than 42 million dollars annually. Because insecticides for corn borer control must be applied over the tops of the corn plants, the threat from drifting insecticides would also be eliminated.

Another benefit that might be derived from reducing or eliminating the use of insecticides for corn borer control is the reduction in the hazard posed to honey bees by conventional chemical insecticides. A reduction in insecticide use should offer more protection for foraging honey bees and their colonies. Further, the *Btk* protein is known to have no activity against honey bee (EPA 1988).

Controlling corn borers with conventional insecticides is not always as effective as growers wish. As stated previously, the primary reason for not achieving acceptable control is inappropriate timing of the insecticide application. Judging when an insecticide application should be made to control corn borers, especially the second generation, is one of the most difficult corn insect management decisions a grower has to ponder. The presence of more than one ecotype confounds the decision-making process. Continuous expression of *Btk* in corn should eliminate management decisions and risks associated with accurate timing of application of conventional insecticides. However, the implementation of IP-corn technology will likely require changes in treatment threshold recommendations.

A reduction in or elimination of the use of conventional insecticides should also have positive benefits within the agricultural industry. Costs of shipping, storage, and handling of conventional insecticides will be reduced. In addition, the cost of development of IP-corn should be less than the cost of development for new chemical insecticides. Finally, the use of fewer insecticides should improve farm worker safety, particularly the safety of consultants who must contend with re-entering fields after they have been treated with insecticides.

Compatibility with other management tactics

IP-corn provides the advantages of both host plant resistance and insecticide efficacy. The *Btk* protein is selectively active against lepidopteran larvae (caterpillars); thus, its presence in corn plants will have no deleterious effects against nontarget insects, including natural enemies of other insect pests. Pilcher & Obrycki (1994) found no significant differences in numbers of lady beetle life stages, lacewing larvae and eggs, and minute pirate found on either transgenic or isogenic corn. However, densities of natural enemies will probably be affected indirectly by the decrease in pest densities as a result of control by IP-corn.

Planting IP-corn should allow for expanded opportunities for biological control because conventional insecticides will not disrupt populations of naturally occurring predators and parasitoids. For example, spraying for control of corn borers in the Great Plains states often results in outbreaks of spider mites because their natural enemies are killed. The use of IP-corn should prevent these secondary pest outbreaks. In addition, because IP-corn affects only lepidopterans, growing IP-corn should allow growers to alternate and/or mix insecticides with different modes of action, i.e., gut poisons (*Btk*) and nerve poisons (conventional insecticides), for control of other pests of corn.

IP-corn is also compatible with more conventional host plant resistance strategies. In fact, it may be possible to pyramid traditional host plant resistance traits with the expression of *Btk*. This strategy has been suggested as one potential strategy for delaying the onset of resistance within the target insect populations.

Potential impacts of insect protected corn on corn pest management programs

Despite the fact that relatively few corn growers attempt to control European corn borers, corn yields should be protected significantly over time if corn borer infestations are controlled. Barrido & Steffey (1995) demonstrated a yield protection benefit of approximately 23 bushels per acre greater in IP-corn than in nontreated non-IP-corn. This yield differential was attributed solely to damage caused by first generation corn borers in the trial. Farmers are not treating for corn borer control because scouting is unpleasant and, at times, difficult, timing of control tactics is difficult, and some modern hybrids tolerate moderate corn borer infestations. When growers observe the yield protection benefit associated with the absence of corn borers, especially during outbreak years, they will realize the importance of both the pest and the new technology for its management.

The greatest impact of IP-corn will likely be felt first in the Great Plains states where treatments are applied more frequently for control of corn borers and yield losses have been estimated to be 3.9 percent even when current control measures are used (Bergman et al. 1985e). However, significant benefits will also be realized in the Corn Belt states (Bergman et al. 1985a), where yield losses have been estimated to be 2.4 percent when current control measures are used, and in the Lake states (Bergman et al. 1985c) where yield losses have been estimated to be 2.0 percent when current control measures are used. In the northeastern and southeastern states, where variations in moth flight activity exacerbate control decisions, the ease of managing corn borers with IP-corn will also have a positive impact (Bergman et al. 1985d; Bergman et al. 1985f).

Effects of insect protected corn on other lepidopteran pests

IP-corn has exhibited a high level of control of European corn borer larvae, but its impact on other lepidopterans has received less attention. MacIntosh et al. (1990) elucidated the specificity and efficacy of purified *Bacillus thuringiensis* proteins against several agronomically important insects. In addition to European corn borers, six other lepidopterans were sensitive to the *Btk* proteins: tobacco hornworm, cabbage looper, tobacco budworm, corn earworm, black cutworm, and beet armyworm. Numerous other researchers have examined the lethal and sublethal effects and the effects on feeding behavior, food consumption, and development of a large array of insect pests including, but not limited to, the beet armyworm, Bertha armyworm, black cutworm, cabbage looper, corn earworm, fall armyworms, southern corn rootworm, sunflower moth, and tobacco budworm. However, the published literature thus far contains only a few references to the effect of other *Bt*-crops on insects that might attack corn.

IP-corn has been field tested against the southwestern corn borer in Kansas (L. Buschman, R. Higgins & P. Sloderbeck, unpublished data), and the results were promising. Because *Btk*-cotton has efficacy against the corn earworm, IP-corn may offer some control of this insect as well. A recent study by Rice & Pilcher (1994) indicated that IP-corn may have some efficacy against newly hatched armyworms and corn earworms but may not have a significant effect against black cutworms and stalk borers.

Effects of insect protected corn on other insect pests of corn

Growing IP-corn will likely have no negative effects on management of several other insect pests of corn, and may have positive effects on management of some. Since IP-corn is unlikely to show any efficacy against some of the soil insect complex, this new technology will have little impact on their management. Positive effects on other insect pests of corn may result if growers and consultants concentrate their scouting efforts for insects and mites that appear in the fields at the same time as both generations of corn borers, e.g., corn rootworm beetles, spider mites, and corn leaf aphids. Although growing IP-corn will not eliminate the need for scouting, the focus of scouting could shift from corn borers to other important pests like corn rootworm beetles in the Corn Belt. As mentioned previously, not using insecticides that might otherwise disrupt populations of natural enemies should allow predators and parasitoids to suppress populations of spider mites and corn leaf aphids.

Conclusion

The development of IP-corn will have a major impact on corn pest management wherever European corn borers are present. The yield protection benefits derived from controlling this key insect pest that is under-scouted and under-treated will have a significant economic impact on corn production. The reduction in or the elimination of the use of insecticides for control of corn borers will offer additional economic benefits. The reduction in the use of insecticides should also reduce the threat to many beneficial insects that may, in turn, suppress other insect pests. Hazards to ground water, surface water, honey bees, and farm workers will diminish.

IP-corn is compatible with other pest management practices, including biological control and host plant resistance, and should have no negative impact on management of the other key pests of corn. The integration of IP-corn with other forms of resistance or tolerance will provide solid footing for the development of nonchemical technologies for other major insect pests. IP-corn may offer additional benefits by controlling other troublesome lepidopteran insect pests.

Although the potential for the development of resistance in corn borers against the *Btk* protein is a real concern, numerous resistance management tactics are being analyzed. Implementation of rational uses of IP-corn with resistance management as a paramount consideration should ensure that the benefits of this new technology in agriculture can be maintained for a long time.

Table 1. Percentage Infestations of European Corn Borer
Data from Illinois, 1986 - 1994

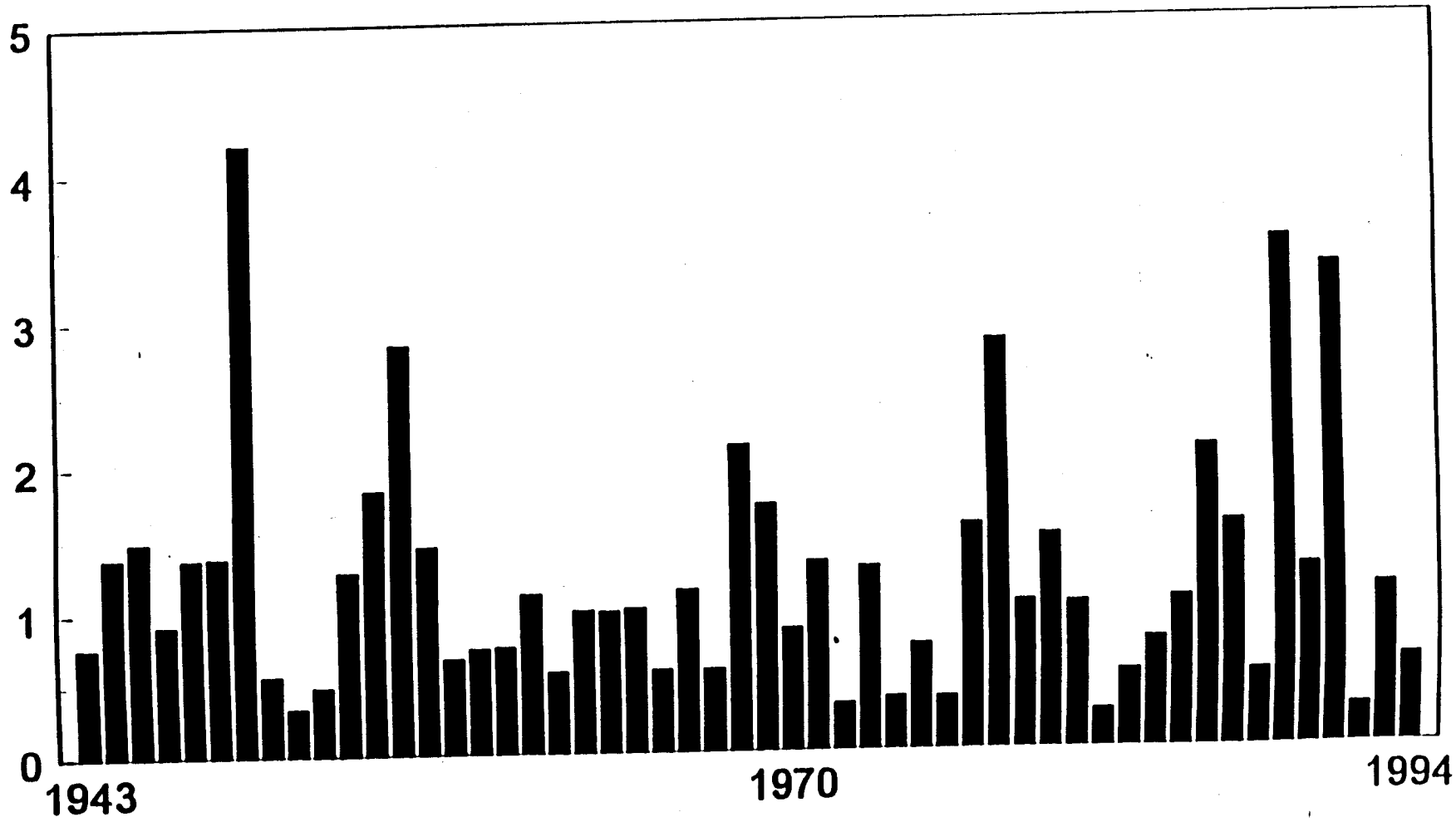
Year	Percentage of stalks infested
1986	80.0
1987	78.0
1988	30.7
1989	78.3
1990	59.5
1991	91.4
1992	30.9
1993	50.3
1994	49.0

Table 2. **Percentage Yield Losses Attributed to European Corn Borers in Six Regions of the United States**[a]

Region (states)	Average percentage yield loss when current controls are used	Average percentage yield loss when no insecticides are used
Corn Belt (IA, IL, IN, OH)	2.4	5.6
Delta (AR, KY, LA, MS, TN)	0.6	3.4
Lake (MI, MN, WI)	2.0	4.5
Northeast (DE, MD, NJ, NY, PA, VA)	1.1	3.7
Northern Plains (CO, KS, NE, ND, SD)	3.9	7.0
Southeast (AL, GA, NC, SC)	2.3	2.6

[a] Data taken from Bergman et al. (1985a-f).

Figure 1. Densities (Larvae per Plant) of 2nd Generation European Corn Borers in Illinois 1943-1994



Management Worksheet for First-Generation Corn Borer

_____ % of 100 Plants Infested x _____ Average No. Borers/Infested Plant = _____ Borers/Plant
(determined by checking whorls from 10 plants)

_____ Borers/Plant x _____ % Yield Loss/Borer* = _____ % Yield Loss

_____ % Yield Loss x _____ Expected Yield (Bu/A) = _____ Bu/A Loss

_____ Bu/A Loss x $ _____ Price/Bu = $ _____ Loss /A

$ _____ Loss/A x _____ % Control = $ _____ Preventable Loss/A
(80% for granules)
(50% for sprays)

$ _____ Preventable Loss/Acre – $ _____ Cost of Control/A =

$ _____ Gain (+) or Loss (–) per acre if treatment is applied

*5% for corn in the early whorl stage; 4% (late whorl); 6% (pretassel).

Management Worksheet for Second-Generation Corn Borer

_____ Number of Egg Masses/Plant x 4 Borers/Egg Mass* = _____ Borers/Plant
(cumulative counts, taken 7 days apart)

_____ Borers/Plant x _____ Yield Loss/Borer** = _____ % Yield Loss

_____ % Yield Loss x _____ Expected Yield = _____ Bu/A Loss

_____ Bu/A Loss x $ _____ Price/Bu = $ _____ Loss/A

$ _____ Loss/A x 75 % Control = $ _____ Preventable Loss/A

$ _____ Preventable Loss/Acre – $ _____ Cost of Control/A =

$ _____ Gain (+) or Loss (–) per acre if treatment is applied

*Assumes survival rate of 20 percent (4 borers/egg mass).
**5% for corn in the early whorl stage; 4% (late whorl); 6% (pretassel); 4% (pollen shedding); 3% (kernels initiated). Use 3% per borer per plant if infestation occurs after silks are brown. The potential economic benefits of treatment decline rapidly if infestations occur after corn reaches the blister stage.

Figure 2. Management Worksheets for First and Second Generation European Corn Borers (from Gray & Steffey 1995).

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APPENDIX II

USDA Final Reports

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

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11/92 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 11/2/92 over an area of 0.035 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 481-11-1, 540-02-2, 523-06-1, 546-09-1, 572-05-1, *576-01-1*, 576-01-2

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 300 ears were harvested 4/8/93 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*

DISPOSAL

Plots were destroyed on approximately 4/30/93 by burning crop residues, followed by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background.

Disease susceptibility

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

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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. At the last observation on 8/93, after four cycles of irrigation, prior to the last disking, only 6 volunteer plants were observed. Given the large number of ears (virtually all non-transgenic) that were disked into the field following harvest, the recurrence of volunteer corn following irrigation is normal.

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

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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

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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

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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.

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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.

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APHIS # 92-209-03
MON# 92-093

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1/93 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 1/12/93 over an area of 0.06 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1, 572-05-1, *576-01-1*, 576-01-2

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

The following lines were selected for harvested on 5/10/93 (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1, *576-01-1*

DISPOSAL

Plots were destroyed on approximately 5/17/93 by disking crop residues.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background.

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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. Approximately 1/3 of the plants were transgenic, and 2/3 non-transgenic. Paired row arrangements of transgenic and non-transgenic lines precluded separation of transgenic volunteers from non-transgenic volunteers. After three cycles of disking and irrigation, no volunteers were observed. This observation is typical for complete stands of non-transgenic plots, also.

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APHIS # 92-209-03
MON# 92-093

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6/93 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 6/8/93 over an area of 0.26 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1, *576-01-1*, *557-04-4*, 540-8-1, 540-02-2, *572-24-1*, 576-12-1

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

The following lines were harvested 9/15/93 (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1, *576-01-1*, *557-04-4*, 540-8-1, 540-02-2, *572-24-1*, 576-12-1

DISPOSAL

Plots were destroyed on approximately 9/22/93 by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 or BC1F1 between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background.

CBI DELETED

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. Approximately 1/3 of the plants were transgenic, and 2/3 non-transgenic. Paired row arrangements of transgenic and non-transgenic lines precluded separation of transgenic volunteers from non-transgenic volunteers. After three cycles of disking and irrigation, no volunteers were observed. This observation is typical for complete stands of non-transgenic plots, also.

APHIS# 92-232-01
MON# 92-097

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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 4/30/93 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)	523-06-1, 572-05-1, *576-01-1*, 576-01-2

Reactions to natural Lepidopteran insect infestations were used to identify positive plants for backcrossing. Successful pollinations were made from selected lines.

The following lines were harvested 7/20/93 (Italicized line(s) indicate lines still being considered for commercialization):

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

DISPOSAL

Plots were destroyed on approximately 7/27/93 by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background.

Disease susceptibility

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

CBI DELETED

Insect susceptibility

The tolerance of positive segregants to Lepidopterous pests was adequate to distinguish positives from negatives. Insecticides were applied to non-transgenic 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

Field was irrigated and disked again to remove germinated volunteer plants. Approximately three weeks after harvest of the corn, the field was planted to soybeans, and subsequent volunteer corn plants removed manually.

APHIS# 92-265-01
MON# 92-126

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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 4/2/93 over an area of 0.02 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1, *576-01-1*

Glyphosate treatment at 16 oz Roundup®/acre was used to identify positive plants for backcrossing. Successful pollinations were made from selected lines. Line 576-01-1 was susceptible to glyphosate, and no pollinations were made from this line.

The following lines were harvested 7/7/93 (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1

DISPOSAL

Plots were destroyed on approximately 8/11/93 by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background. Four stunted plants of lines 523-06-1 and 546-09-1 were observed.

Disease susceptibility

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

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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. Three cycles of irrigation and field disking were used to control volunteers; 8/11/93, 9/14/93, and 10/15/93. There were no volunteer plants following the last cycle.

APHIS# 92-265-01
MON# 92-126

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7/93 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 7/15/93 over an area of 0.07 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1

Glyphosate treatment at 16 oz Roundup®/acre was used to identify positive plants for backcrossing. Successful pollinations were made from selected lines.

The following lines were harvested 10/12/93 (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 546-09-1

DISPOSAL

Plots were destroyed on approximately 11/16/93 by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an early backcross between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background.

Disease susceptibility

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

CBI DELETED

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. Three cycles of irrigation and field disking were used to control volunteers; 11/16/93, 12/15/93, and 1/10/94. There were no volunteer plants following the last cycle.

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

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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

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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 Jersevville, 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

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

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.

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

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.

APHIS# 93-021-05
MON# 93-010R

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5/93 Planting

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

Observation of efficacy of lines with the Bt gene in control of European Corn Borer (ECB) and seed increase of selected lines.

EXPERIMENT DESCRIPTION

The following lines were planted on 5/18/93 over an area of 0.1 acres:

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	523-06-1, 557-04-4, 572-24-1, 540-02-2, 540-08-1, 576-12-1, 546-09-1, *572-16-1*, *576-01-1*

Glyphosate and ECB's were used to identify positive plants for backcrossing. Successful pollinations were made from selected plants of all lines except dave. Only seeds from 572-24-1 were harvested. Harvest was 9/23/93 - 9/24/93. Bt lines fully controlled ECB in these trials.

DISPOSAL

Plots were destroyed by approximately 10/7/93 by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were still segregating for many traits. Variability in plant phenotype was consistent with this genetic background.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to recurrent parent plants.

Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to recurrent parent plants.

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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

The field was monitored for volunteers during November, 1993, and April and May, 1994. No volunteer corn was noted.

APHIS# 93-021-06
MON# 93-011R

CBI DELETED

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93 Corn Belt Plantings

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

- Evaluate efficacy of Bt in controlling European Corn Borer (ECB)
- Generate seed for further testing and evaluation

EXPERIMENT DESCRIPTION and RESULTS

The following lines were planted on 6/18/93 over an area of 0.15 acres:

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	557-04-4, 540-02-2, 576-12-1, 540-08-1, 572-24-1, 523-06-1, 576-01-1, 546-09-1, 572-16-1, 576-01-2, 572-05-1

Positive plants were identified using results of artificial ECB infestations. Where the Bt gene was expressing, lines showed less ECB damage than susceptible checks.

The following lines were harvested on 11/2/93:

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	557-04-4, 540-02-2, 576-12-1, 540-08-1, 572-24-1, 523-06-1, 576-01-1, 546-09-1, 572-16-1, 576-01-2, 572-05-1

Plots were destroyed 11/15/93 by disking and plowing. Grain not saved was ground up and broadcast over the field.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 or early backcross between Hi-Type II and B73 or other elite inbred line. Variability in plant phenotype was consistent with these genetic backgrounds.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to nontransgenic plants of similar genotype.

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Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to nontransgenic plants of similar genotype.

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

Field was observed 7/5/93, after most volunteer seed should have germinated, but prior to flowering. No volunteer corn was observed in the soybeans now planted at the site.

APHIS# 93-021-07
MON# 93-012R

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5/93 Planting

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

- Evaluate efficacy of Bt lines to European Corn Borer (ECB)
- Generate seed for further testing

EXPERIMENT DESCRIPTION

The following lines were planted on 5/15/93 over an area of 0.1 acre (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01	557-04-4, 540-02-2, 546-09-1, 572-24-1, 540-08-1
(PV-ZMBK07+PV-ZMGT10)	523-06-1, *576-01-1*, *572-16-1*, 576-12-1

ECB was used to identify positive plants for backcrossing.

Successful pollinations were made from selected lines. All lines evaluated controlled ECB.

The following lines were harvested 11/15/93 (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01	557-04-4, 540-02-2, 546-09-1, 572-24-1, 540-08-1
(PV-ZMBK07+PV-ZMGT10)	523-06-1, *576-01-1*, *572-16-1*, 576-12-1

DISPOSAL

Plots were destroyed on approximately 11/22/93 by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 or early backcrosses between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to recurrent parent plants.

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Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to recurrent parent plants except as noted for the ECB efficacy trial.

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

The field was planted to annual ryegrass. In the area of the field that was planted to BT and recurrent parents, there were approximately 100 volunteer corn plants. These were removed by mowing prior to folowering.

APHIS# 93-021-08
MON# 93-013R

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93 Corn Belt Plantings

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

- Evaluate efficacy of Bt in controlling European Corn Borer (ECB)
- Produce seed for subsequent breeding and evaluations

EXPERIMENT DESCRIPTION and RESULTS

The following lines were planted on 5/27/93 over an area of 0.03 acres (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, *557-04-4*, 540-08-1, 576-12-1, 546-09-1, *576-01-1*, *572-24-1*, 540-02-2

Reactions to natural Lepidopteran insect infestations and/or reaction to post-emergent applications of glyphosate were used to identify positive plants for backcrossing. Successful pollinations were made from selected lines.

The following lines were harvested 10/25/93 (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	*572-16-1*, 523-06-1, 557-04-4, 540-08-1, 576-12-1, 546-09-1, *576-01-1*, 572-24-1, 540-02-2

Plots were destroyed 11/8/93 by disking and plowing.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and B73. Variability in plant phenotype was consistent with these genetic backgrounds. In comparisons between lines and adjacent elite inbred lines, no differences were noted in germination or in seedling growth under the excessive rainfall conditions prevailing during these growth stages. No differences were observed between transgeic and non-transgenic lines with respect to pollen-shed, silk extrusion, or seed set on hand-pollinated ears.

Disease susceptibility

An unusually high incidence of anthracnose leaf blight and rust were present. No differences were noted in the incidence of these foliar diseases on transgenic plants compared to nontransgenic 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.

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

In observations made during April and May, 1994, no volunteer corn was present. The field is currently planted to soybeans, and Treflan® and Sencor® have been applied for weed control.

APHIS# 93-021-04
MON# 93-014R

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FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVES

- Assess the efficacy of Bt gene against European Corn Borer (ECB)
- Assess level of tolerance to Roundup® herbicide
- Introgress transgenes into elite inbred lines through traditional backcross breeding techniques.

EXPERIMENT DESCRIPTION

The following lines were planted in these experiments: (Italicized line(s) indicate lines still being considered for commercialization):

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	523-06-1, 546-09-1, *576-01-1*

Planting dates and acres planted were:

5/13/93 and 6/7/93, 0.01 acre planted for ECB efficacy testing
5/26/93, 0.004 acre planted for gene introgression
6/7/93, 0.025 acre planted for Roundup® tolerance evaluations

PCR or glyphosate treatments were used to identify positive plants.

The following lines were harvested during the period 9/10/93 - 9/13/93 :

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	523-06-1, 546-09-1

RESULTS

Bt efficacy: Under artificial infestation, lines exhibited almost complete protection against first generation ECB, averaging less than a rating of 2 on a 1(no damage) - 9 (severe damage) scale. Lines provided similar protection from stalk tunnelling, with all lines averaging less than 0.3 inches of tunnels.

Breeding nursery: Desired crosses were made.

Roundup tolerance: 576-01-1 was very susceptible. The other two lines showed some stunting and flowering delays or infertility.

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DISPOSAL

Plots were destroyed on approximately 11/15/93 by disking.

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and elite inbred lines. Variability in plant phenotype was consistent with this genetic background.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to recurrent parent plants.

Insect susceptibility

Other than experimental results, no differences were noted in the frequency or severity of insect damage of transgenic plants compared to recurrent parent plants.

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

No volunteers were noted in the fall following disking. The following spring the fields were planted to soybeans, and any volunteer corn was removed using herbicides, cultivation or manual weed control. The number of volunteer plants observed, although not counted, was consistent with that normally seen with complete stands of non-transgenic corn.

The Minnesota location identified in the original permit was not established.

APHIS# 93-021-03
MON# 93-015R

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93 Corn Belt Plantings

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

- Evaluate efficacy of Bt genes in controlling European Corn Borer (ECB)
- Assess yield differences
- Generate plant material for regulatory testing

EXPERIMENT DESCRIPTION and RESULTS

Regulatory: Lines 523-06-1 and 576-01-1, both containing PV-ZMCT01 (PV-ZMBK07 + PV-ZMGT10), were planted in these trials. Transgenic acreage at each of the two locations was approximately 0.07 acres. Planting and harvest dates for each of the locations were:

LOCATION	PLANTED	HARVESTED
1	5/20/93	9/30/94
2	5/17/93	Flooded initiated 7/9, plots totally submerged 7/11, plots completely destroyed 7/20/94

Adequate plant tissue and seed were obtained from these locations to conduct required analyses.

Yield and efficacy: Lines 523-06-1, 576-01-1, 546-09-1, and 572-16-1 were included in the yield trial and efficacy trial, and line 540-02-2 was included in the efficacy trial only. These trials were planted both at locations 1 and 2 on the same dates as the regulatory trials. Acreage at each site was approximately 0.32. The plots at location 2 were flooded prior to pollination as described above. Harvest at location 1 was 10/28/93.

In the efficacy trial, all Bt lines controlled ECB. In the yield trial, there was no significant difference in yield between BT+ and BT- populations of lines 576-01-1, 546-09-1, or 572-16-1. There were indications that the BT+ population of line 523-06-1 had lower yields than the BT- population of the same line.

DISPOSAL

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

CBI DELETED

GENERAL FIELD OBSERVATIONS

Genetically, lines were an F1 between Hi-Type II and B73, or an early backcross to elite inbred lines per se or top crossed to a tester inbred line. Variability in plant phenotype was consistent with these genetic backgrounds.

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to nontransgenic plants of similar genotype.

Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to nontransgenic plants of similar genotype.

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

Currently, field is planted to oats. No volunteer corn was observed during May, 1994 or June, 1994 observations.

APHIS# 93-060-06
MON# 93-044R

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93 Corn Belt Plantings

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

OBJECTIVE

Generate plant material for regulatory testing

EXPERIMENT DESCRIPTION and RESULTS

Regulatory: Lines 523-06-1 and 576-01-1, both containing PV-ZMCT01 (PV-ZMBK07 + PV-ZMGT10), were planted in these trials. Transgenic acreage at each of the two locations was approximately 0.07 acres. Planting and harvest dates for each of the locations were:

LOCATION	PLANTED	HARVESTED
3	5/17/93	10/1/93
4	5/13/93	10/1-93

Adequate plant tissue and seed were obtained from these locations to conduct required analyses.

DISPOSAL

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

GENERAL FIELD OBSERVATIONS

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

Disease susceptibility

No differences were noted in disease incidence of transgenic plants compared to nontransgenic plants of similar genotype.

Insect susceptibility

No differences were noted in the frequency or severity of insect damage of transgenic plants compared to nontransgenic plants of similar genotype.

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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

The fields at both locations are currently planted to soybeans. Fields have been monitored biweekly for corn volunteers since May 1, and any volunteer corn removed by hand or using herbicides. There were no volunteer corn plants in either field to date.

CBI DELETED

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 [CBI DELETED]

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 [CBI DELETED].

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 [CBI DELETED].

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.

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SUMMARY REPORT OF FIELD TEST DATA

Approved USDA Ref. Numbers: 93-146-02N
Monsanto Ref. Number: 93-070RA

PURPOSE

Introgress the Bt gene into elite germplasm through several cycles of backcrossing.

GENERAL RESULTS OF EXPERIMENT

This permit covered 3 planting at the [CBI DELETED
]

The first planting occurred on December 2, 1993. Total planted acreage was 1.0 transgenic acres and 1.8 total acres. Line numbers included in this planting were:

PV-ZMCT01 523-06-1, 576-01-1, 546-09-1, and 572-16-1.
(PV-ZMBK07 + PV-ZMGT10)

Each of these lines was backcrossed to their recurrent parent -- an elite inbred. Also, the lines were crossed to elite inbreds to make experimental hybrids. The seed was harvested from March 29 thru April 13, 1994. A portion of the backcross material was sent to the facility at [CBIDELETED] and the remainder was replanted in Hawaii in the next planting. All of the crossing seed was sent to [CBIDELETED] for yield testing.

The second and third plantings occurred on 3/17/94 and 4/11/94. All lines continued to be backcrossed to their recurrent parents and the most advanced material was self pollinated in this planting. The seed was harvested on 6/28/94 and 7/8/94 respectively. A portion of the seed was returned to the facility at [CBIDELETED] 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.

CBI DELETED

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 that 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.

CBI DELETED

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 [CBI DELETED]

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 [CBIDELETED] and the remainder was replanted in Hawaii in the next planting. All of the crossing seed was sent to [CBIDELETED] 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.

CBI DELETED

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 [CBI DELETED] 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.

LOXLEY, ALABAMA PROGENY TEST GROWOUT
OCTOBER, 1993 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-250-04N
Monsanto: 93-101R

EXPERIMENT DESCRIPTION

The objective of the experiment was to determine segregation of reaction to over the top applications of Roundup® of F3 and test-crossed F2 families of lines containing genes conferring tolerance to glyphosate.

Plots were located in Loxley, Alabama (Baldwin County). The following lines were planted on 10/12/93 over an area of 1.4 acres (1.7 acres if alleys are included).

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	588-13-1,523-06-1,572-24-1,576-01-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2

Roundup® at 24 oz/acre was applied 10/15/93. Plots were evaluated for segregation (all tolerant, segregating, all susceptible) on 11/8/93, and selected plots were re-evaluated 11/15/93.

Plots were not taken to flower. All plots were destroyed 11/20/93.

The isolation method utilized was plants were not allowed to reach reproductive maturity.

RESULTS

Families were identified which were homozygous for the gene based on segregation to glyphosate. Remnant seed from these families were used in seed production at other sites for use in 1994 field trials.

GENERAL FIELD OBSERVATIONS

The short duration of the experiment, and use of systemic insecticides resulted in only a single observation for deviant characteristics possible. No evidence of atypical insect or disease susceptibility was noted, nor did the plants exhibit any unusual growth or other phenotypic characteristics.

DISPOSAL

Plots were destroyed on 11/20/93 by disking.

VOLUNTEERS

No volunteers noted prior to crop rotation in 1994.

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.

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

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			

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.

CBI DELETED

[CBI DELETED], Molokai (Maui County), HI]
OFF-SEASON TESTING AND BREEDING NURSERY
DECEMBER, 1993 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 produce seed for testing during the 1994 mainland U.S. growing season and to introgress the genes of interest into proprietary inbred lines.

The trial was planted 12/9/93 over an area of 0.21 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, 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 eliminated volunteer plants.

CBI DELETED

[CBI DELETED], 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.

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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
	599-04-2, 599-04-3, 604-09-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	762-03-1
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	766-07-1
PV-ZMCT10 (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.

C B I DELETED

[CBI DELETED], 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.

CBI DELETED

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.

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 Mr. Peter Eichhorn, Hawaiian Research Ltd.

RESULTS

The trial was planted on three dates, December 6th, 8th, and 10th of 1993, at Hawaiian Research Ltd. 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	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			

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.

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[CBI DEL], 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: 93-288-01N
Monsanto: 93-146RA

EXPERIMENT DESCRIPTION

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

The trial was planted 5/20/94 over an area of 0.005 acres (excluding alleyways). The table below lists the lines planted, arranged by vector. Plots were harvested 8/31/94.

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	576-01-1

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

RESULTS

Seed production was as expected under Hawaiian conditions.

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

Three cycles of irrigation and cultivation eliminated volunteer plants.

CBI DELETED

[CBI DEL], Molokai (Maui County), HI
OFF-SEASON TESTING AND BREEDING NURSERY
September, 1994 PLANTING

FINAL REPORT

Gregory B. Parker
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 93-288-01N
Monsanto: 93-146RA

EXPERIMENT DESCRIPTION

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

The trial was planted 9/26/94 over an area of 0.011 acres (excluding alleyways). The table below lists the lines planted, arranged by vector. Plots were harvested 1/12/95.

Vector	Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	576-01-1

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

RESULTS

Seed production was as expected under Hawaiian conditions.

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

Three cycles of irrigation and cultivation eliminated volunteer plants.

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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.

CBI DELETED

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.

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[CBI DELETED]

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.

CBI DELETED

Vector	Lines
PV-ZMBK10	631-03-1
PV-ZMBK12L	749-01-1
PV-ZMBK16L	754-07-4, 754-10-1
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-04-2, 559-39-2, 572-16-1, 572-24-1, 574-04-2, 575-07-2, 581-07-1, 591-03-2
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. 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.

CBI DELETED

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CBI [CBI DELETED]

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.

CBI DELETED

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.

CBI DELETED

[CBI DELETED] [CBI DELETED] 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.

CBI DELETED

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, 523-09-1, 540-04-1, 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 (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
PV-ZMCT04 (PV-ZMGT13+PV-ZMGT05)	425-01-2, 425-02-1
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	462-03-2
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	751-13-1, 762-03-1
PV-ZMCT08 (PV-ZMBK07+PV-ZMGT01)	694-02-1, 694-06-1, 767-07-1
PV-ZMCT09 (PV-ZMBK23+PV-ZMGT01)	768-06-1
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 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.

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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.

CBI DELETED

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	
	599-04-2,	599-04-3,	604-09-1		
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2				
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	762-03-1				
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	766-07-1				
PV-ZMCT10 (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.

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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.

CBI DELETED

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.

CBI DELETED

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: [CBI DELETED].

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 [CBI DELETED] IA.

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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, 658-06-1 599-04-2, 599-04-3, 604-09-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	762-03-1
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	423-06-1
PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	766-07-1
V-ZMCT10 (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
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: 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 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
	599-04-2, 599-04-3, 604-09-1
PV-ZMCT02	
(PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05	
(PV-ZMBK13+PV-ZMGT05)	762-03-1
PV-ZMCT06	
(PV-ZMBK17+PV-ZMGT01)	423-06-1
PV-ZMCT07	
(PV-ZMGT03+PV-ZMGT05)	766-07-1
V-ZMCT10	
(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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[CBI DELETED] (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.

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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

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	572-16-1, 572-24-1,576-01-1, 581-07-1, 639-02-1,
(PV-ZMBK07+PV-ZMGT10)	658-06-1
PV-ZMCT02	599-04-2, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	

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.

CBI DELETED

[CBI DELETED] (Maui County) Maui, Hawaii
OFF-SEASON BREEDING NURSERY
May, 1994 PLANTING

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-074-11N phenotype: Lepidopteran insect resistance
MONSANTO: 94-069R

EXPERIMENT DESCRIPTIONS

The objective of this planting was to introgress the genes of interest (GOI) into elite inbred line backgrounds.

The transgenic lines planted in these trials are listed below. The trial was planted 5/18/94 and harvested at maturity. Plots were isolated at least 200 meters from any other corn not part of these trials, and tassels were bagged. Total transgenic area of the trials was 0.017 acres.

Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10): 576-01-1, 658-06-1
PV-ZMBK16L: 748-04-3

RESULTS

Adequate seed was produced to initiate the next cycle of backcrossing. Seed production was consistent with Hawaii conditions.

GENERAL FIELD OBSERVATIONS

Transgenic lines were compared to related non-transgenic inbreds. 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

Volunteers were controlled using cycles of irrigation and mechanical destruction of resulting plants.

CBI DELETED

[CBI DELETED] (Champaign County) , IL
1994 CORN BELT TRIAL

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-074-11N phenotype: Lepidopteran insect resistance
MONSANTO: 94-069XRA

EXPERIMENT DESCRIPTIONS

The objective of this planting was to assess the efficacy of the *Btk* gene in controlling European corn borer (ECB).

The transgenic lines planted in these trials are listed below. The trial was planted 5/18/94 and harvested at maturity. Plots were isolated at least 200 meters from any other corn not part of these trials. Total transgenic area of the trial was 0.14 acres.

Lines
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10): 576-01-1, 658-06-1
PV-ZMBK16L: 748-04-3

RESULTS

All three lines exhibited commercial control of ECB.

GENERAL FIELD OBSERVATIONS

Transgenic lines were compared to related lines not containing the *Bt* gene. No differences were noted in disease or insect susceptibility, except for control of ECB. 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 was initiated at harvest and plot destruction in 1994 and is continuing into the spring 1995 season.

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[CBI DELETED]
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.

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Vector	Lines
PV-ZMBK12L	749-01-1
PV-ZMBK16L	754-07-4, 748-04-3
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 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, 599-04-3cal, 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

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).

CBI DELETED

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[CBI DELETED]
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 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) evaluated for tolerance to different application regimes of Roundup® herbicide; and (3) evaluated for tolerance to feeding damage from European corn borer (ECB).

The trials were planted May 20, 1994, over an area of 0.6 acres. The table below lists the lines planted, arranged by vector.

Selected plants and plots were mechanically harvested October 27, 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.

Vector	Lines
PV-ZMBK12L	749-01-1
PV-ZMBK16L	754-07-4, 748-04-3
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 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

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 in 1994 after the field season and will conclude this spring (1995).

CBI DELETED

[CBI DELETED] (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.

CBI DELETED

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

CBI DELETED

[CBI DELETED] (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: 94-072XRA
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	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, 576-01-1, 581-07-1ner,
	591-03-2, 639-02-1, 658-06-1
PV-ZMCT02	599-04-2, 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-ZMCT10	766-07-1
(PV-ZMBK23+PV-ZMGT10)	

CBI DELETED

[CBI DELETED] (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
	599-04-2, 599-04-3, 604-09-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	762-03-1
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	766-07-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	

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-ZMBK10I
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

CBI DELETED

[CBI DELETED]
[CBI DEL.] (Hamilton County), NE
[CBI DELETED] Washington County), IA
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-082-09N phenotype: Lepidopteran insect resistance
94-087-12N phenotype: Glyphosate tolerance

Monsanto: 94-081XRA
94-104XRA

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 within the same testing block at these locations.

The trials were planted May 20, 1994, covering 0.04 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 or planted after any plants within 200 meters would have receptive silks.

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.

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DISPOSAL

Following harvest, plant residue was disked into the soil.

VOLUNTEERS

Monitoring for volunteers was initiated in 1994 at harvest 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, 754-10-1
PV-ZMCT01	572-16-1, 572-24-1, 576-01-1, 581-07-1, 639-02-1,
(PV-ZMBK07+PV-ZMGT10)	658-06-1
PV-ZMCT02	599-04-2, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	

CBI DELETED

[CBI DELETED] (Champaign) County, IL
1994 CORN BELT TRIALS

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-082-09N phenotype: Lepidopteran insect resistant
94-087-12N phenotype: Glyphosate tolerant

Monsanto: 94-081XRA
94-104XRA

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 tested in the same testing block location.

The trials were planted May 31, 1994, covering 0.15 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 or planted after any plants within 200 meters would have receptive silks.

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

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.

VOLUNTEERS

Monitoring for volunteers was initiated after harvest and monitoring 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, 540-04-1c, 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

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[CBI DELETED] [CBI DELETED] 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.

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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	481-10-1, 513-11-2, 523-06-1, 540-04-1, 544-03-2,
(PV-ZMBK07+PV-ZMGT10)	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	599-04-2, 599-04-3, 604-09-1
(PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05	
(PV-ZMBK13+PV-ZMGT05)	62-03-1
PV-ZMCT06	
(PV-ZMBK17+PV-ZMGT01)	402-08-1
PV-ZMCT07	
(PV-ZMGT03+PV-ZMGT05)	766-07-1
PV-ZMCT10	
(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 ground up and plowed into the soil after harvest.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into 1995.

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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.

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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	
	599-04-2,	599-04-3,	604-09-1		
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2				
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	62-03-1				
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	766-07-1				
PV-ZMCT10 (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.

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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.

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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 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 523-06-1, 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, 658-06-1 599-04-2, 599-04-3, 604-09-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	62-03-1
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	766-07-1
PV-ZMCT10 (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.

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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 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 523-06-1, 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, 658-06-1 599-04-2, 599-04-3, 604-09-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	62-03-1
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	766-07-1
PV-ZMCT10 (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.

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[CBI DELETED] [CBI DELETED] 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.

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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	
	599-04-2,	599-04-3,	604-09-1		
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2				
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	62-03-1				
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	766-07-1				
PV-ZMCT10 (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.

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[CBI DELETED]
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.

CBI DELETED

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 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 523-06-1, 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)	62-03-1
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 burned.

VOLUNTEERS

Monitoring for volunteers was initiated after the 1994 field season and is continuing into the 1995 spring season.

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CBI [CBI DELETED] (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.

CBI DELETED

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.

CBI DELETED

[CBI DELETED] (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.

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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 (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, 639-02-1, 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

CBI DELETED

[CBI DELETED] (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.

CBI DELETED

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 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 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

CBI DELETED

[CBI DELETED] (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.

CBI DELETED

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, 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

CBI DELETED

[CBI DELETED] (Hall County), NE
1994 CORN BELT TRIAL

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-083-03N phenotype: Lepidopteran insect resistance
Monsanto: 94-089XRA

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

CBI DELETED

[CBI DELETED] (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.

CBI DELETED

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 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 513-11-2, 540-04-1, 544-03-2, 554-03-2, 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 (PV-ZMBK15+PV-ZMGT03)	599-04-2, 599-04-3, 600-14-2, 604-09-1
PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	423-06-1

CBI DELETED

[CBI DELETED] (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: 94-090XRA
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.

VOLUNTEERS

Monitoring for volunteers was initiated at the time of harvest and is continuing into the spring 1995 field 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, 540-04-1, 544-03-2, 554-03-2, 557-04-4, 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-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	423-06-1
PV-ZMCT10 (PV-ZMBK23+PV-ZMGT10)	766-07-1

MONSANTO AG. COMPANY
SEED AND SILAGE PRODUCTION

FINAL REPORT

Kent A. Croon
The Agricultural Group of Monsanto

PERMIT NUMBERS

USDA: 94-105-014N Lepidopteran insect resistance
Monsanto: 94-120XRA

EXPERIMENT DESCRIPTION

The objective of the planting was to produce silage and corn grain under field conditions to support regulatory experiments.

The trial (3.0 acres, excluding alleyways) was planted at the Monsanto - Dardenne Technical Center, O'Fallon, MO (St. Charles county) on 5/23/94. The only corn line planted in this trial was 576-01-1. Silage from the trial was harvested on 9/9/94 and seed was harvested on 11/17/94

A 200 M isolation distance was utilized. No non-transgenic corn which could enter commerce was planted within this distance.

RESULTS

Seed and tissue production was as expected under conditions present at the site.

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.

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 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 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.

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

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-279-03N phenotype: Lepidopteran insect resistance
94-279-04N phenotype: Glyphosate tolerant

Monsanto: 94-203XRA
94-204XRA

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 200 M isolation.

Plots were located on Molokai, Hawaii (Maui County). The trial was planted 11/9/94 over an area of 0.22 acres (excluding alleyways). The table on the next page lists the lines planted, arranged by vector.

Selected plants and plots were hand harvested 3/4-8/95, 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-ZMBK10L	958-12-2, 997-21-5
PV-ZMBK12L	749-01-1, 992-09-1
PV-ZMBK16L	748-04-3
PV-ZMBK20L	1016-03-1, 1032-06-14, 1032-19-14
PV-ZMGT18L	1112-02-1, 1163-07-2
PV-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 540-04-1, 559-39-2, 574-04-2, 575-07-2, 576-01-1, 591-03-2, 658-06-1 599-04-2, 599-04-3, 604-09-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	

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. A relatively severe infestation of rust came in late season. Bacterial leaf blight was also severe in some plots, particularly those in Mo17 background. There appeared to be no difference in the non-transgenic inbred line, Mo17, and lines being backcrossed into Mo17. The bacteria apparently occurs naturally in the irrigation water and certain inbred line backgrounds are more susceptible to it than others. Southern leaf blight was also 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

In progress. The plot area will be irrigated to stimulate germination of volunteers followed by tillage to remove the plants identified. This procedure will be repeated until no volunteers are present.

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.

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, 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, 658-06-1 599-04-2, 599-04-3, 604-09-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	462-03-2
PV-ZMCT05 (PV-ZMBK13+PV-ZMGT05)	762-03-1
PV-ZMCT06 (PV-ZMBK17+PV-ZMGT01)	423-06-1
PV-ZMCT07 (PV-ZMGT03+PV-ZMGT05)	766-07-1
V-ZMCT10 (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.

PUERTO RICO OFF-SEASON TESTING AND BREEDING NURSERY
NOVEMBER, 1994 NURSERY

FINAL REPORT

Gregory B. Parker

PERMIT NUMBERS

USDA: 94-279-03N phenotype: Lepidopteran insect resistance
94-279-04N phenotype: Glyphosate tolerant

Monsanto: 94-203XRA
94-204XRA

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 identified below.

The trial was established at the Monsanto Research Farm in Santa Isabel, Puerto Rico, near Ponce. The trial was planted 11/18/94 over an area of 0.07 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-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-ZMCT01 (PV-ZMBK07+PV-ZMGT10)	481-10-1, 540-04-1, 572-16-1, 574-04-2, 576-01-1, 591-03-2, 658-06-1
PV-ZMCT02 (PV-ZMBK15+PV-ZMGT03)	599-04-2

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 has been initiated. The plot area will be irrigated and volunteers destroyed with tillage. This procedure will be repeated until all volunteers are destroyed.

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
Marsnall	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.

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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 Princeton, 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 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.

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 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 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: 94-024-07N
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 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 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 return to the plot for soil composting.

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.

APPENDIX III

EXAMPLE FIELD MONITORING FORMS

Rcvd 1/20/95

CBI DELETED

USDA Agronomic Check-Off List
B.t./Roundup Ready Corn

Cooperator: [CBI DELETED] Monsanto Ref. No. 94-C99XRA

City and State: Slater, IA

Study Title/Description: Btk Efficacy Trial Date Study Planted: MAY 17, 1994
Terminated: October 28, 1994

Disease Susceptibility: Do transgenic plants have a higher incidence of disease than nontransgenic plants? If yes, to which diseases?

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Specific Diseases/Comments
6-15		✓			
7-25		✓			
8-26		✓			
9-28		✓			

Insect Susceptibility: Do transgenic plants have a higher incidence of nontarget insect species than nontransgenic plants? If yes, which species are more prevalent?

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Specific Insects/Comments
6-15		✓			
7-25		✓			
8-26		✓			
9-28		✓			

Plant Growth Characteristics: Is there a difference in the general appearance and growth of transgenic and nontransgenic plants? If yes, describe the differences.

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Comments
6-15		✓			
7-25		✓			
8-26		✓			
9-28		✓			

Weediness Characteristics: Is the germination of transgenic plants in any way different from nontransgenic plants? If yes, describe differences and potential causes.

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Comments
6-15		✓			
7-25		✓			
8-26		✓			
9-28		✓			

Rcvd
11/20/95

CBI DELETED

USDA Agronomic Check-Off List
B.t./Roundup Ready Corn

Cooperator: [CBI DELETED]

Monsanto Ref. No. 94-089XRA

City and State: Wood River, NE

Study Title/Description: Btk Efficacy Trial

Date Study Planted: May 20 1994
Terminated: Oct 26 1994

Disease Susceptibility: Do transgenic plants have a higher incidence of disease than nontransgenic plants? If yes, to which diseases?

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Specific Diseases/Comments
7/5		No	0	0	Some rust, spotty in scattered areas
7/25		No			Common smut throughout
8/25		No			
9/14		No			

Insect Susceptibility: Do transgenic plants have a higher incidence of nontarget insect species than nontransgenic plants? If yes, which species are more prevalent?

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Specific Insects/Comments
7/5		No	0	0	
7/25		No			
8/25		No			
9/14		No			

Plant Growth Characteristics: Is there a difference in the general appearance and growth of transgenic and nontransgenic plants? If yes, describe the differences.

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Comments
7/5		No	0	0	
7/25		No			
8/25		No			
9/14		No			

Weediness Characteristics: Is the germination of transgenic plants in any way different from nontransgenic plants? If yes, describe differences and potential causes.

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Comments
7/5		No	0	0	
7/25		No			
8/25		No			
9/14		No			

--CBI DELETED

RECEIVED
SEP 23 1994

USDA Agronomic Check-Off List
B.t./Roundup Ready Corn

V

Cooperator: [CBI DELETED]

Monsanto Ref. No. 94-085xRA

City and State: Champaign, IL

Study Title/Description: B.t./RR Nursery

Date Study Planted: May 18, 1994

Disease Susceptibility: Do transgenic plants have a higher incidence of disease than nontransgenic plants? If yes, to which diseases?

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Specific Diseases/Comments
5/26	N/A		—	—	—
6/30		X	—	—	—
7/29		X	—	—	—
8/31		X			
9/16 Harvest		X			

Insect Susceptibility: Do transgenic plants have a higher incidence of nontarget insect species than nontransgenic plants? If yes, which species are more prevalent?

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Specific Insects/Comments
5/26		X	<1%	<1%	Some Black Cutworm present in non-transgenic lines
6/30		X			
7/29		X			
8/31		X			
9/16 Harvest		X			

Plant Growth Characteristics: Is there a difference in the general appearance and growth of transgenic and nontransgenic plants? If yes, describe the differences.

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Comments
5/26		X			
6/30		X			
7/29		X			
8/31		X			
9/16 Harvest		X			

Weediness Characteristics: Is the germination of transgenic plants in any way different from nontransgenic plants? If yes, describe differences and potential causes.

Date	Yes	No	%of plants affected (in transgenic line)	% of plants affected (in non-transgenic line)	Comments
5/26		X			emergence 6 days after planting, normal appearance. No double embryos observed. Vigor consistent with stage of inbreeding
6/30		X			
7/29		X			

USDA Agronomic Check-Off List
B.t./Roundup Ready Corn

perator: Hawaiian Research Ltd

~~Monsanto Ref. #~~ [illegible]
USDA Ref. # 93-308-02N
93-308-04N

y and State: Kaunakakai, Hawaii

Date Study Planted: /12/10/93

dy Title/Description: Rainbow Resistance / BT.
- for Maissoour

sease Susceptibility: Do transgenic plants have a higher incidence of disease than non transgenic plants?
yes, to which diseases?

Date	Yes	No	%of plants observed	% of plants affected	Specific Diseases/Comments
1/10/94		✓			No noticeable difference was seen between non and Transgenic plants. The plot showed the normal disease pressure we would expect from Northern and Southern Leaf blights and from bacterial Leaf blight.
2/15/94		✓			
3/12/94		✓			

sect Susceptibility: Do transgenic plants have a higher incidence of nontarget insect species than nontransgenic plants? If yes, which species are more prevalent?

te	Yes	No	%of plants observed	% of plants affected	Specific Insects/Comments
10/94		✓			No noticeable difference was seen between non and Transgenic plants. The plot showed the normal pressure from the nontarget insects that we usually see.
15/94		✓			
12/94		✓			

Plant Growth Characteristics: Is there a difference in the general appearance and growth of transgenic and nontransgenic plants? If yes, describe the differences.

Date	Yes	No	%of plants observed	% of plants affected	Comments
1/10/94		✓			
2/15/94		✓			
3/12/94		✓			

Weediness Characteristics: Is the germination of transgenic plants in any way different from nontransgenic plants? If yes, describe differences and potential causes.

Date	Yes	No	%of plants observed	% of plants affected	Comments
1/10/94		✓			
2/15/94		✓			

BT CORN USDA AGRONOMIC CHECK-OFF

X = normal variety characteristics

O = off-type, resistant, or otherwise different from variety in any way

		Physical character.								Disease Suscept.						Insect Suscept.						
Entry	Rep	Plant vigor	Stunted growth/Plant ht.	Chlorotic color	Flowering	Tasseling (early, late)	Others?	# of Plants observed	% Plants affected (if any)	Leaf blight (describe)	Stalk rot (describe)	Ear rot (describe)	Others?	# of Plants observed	% Plants affected (if any)	Diabrotica spp. (describe)	Noctuidae spp. (describe)	Spider mites	Others?	# of Plants observed	% Plants affected (if any)	Comments/description
																						Transgenic plants were
																						not noticeably different
																						from non-transgenic
																						for the above characteristics

Date: 2-7-94 Trial #: ________ Cooperator: Hawaii Continuous Nursery

BT CORN USDA AGRONOMIC CHECK-OFF

X = normal variety characteristics

O = off-type, resistant, or otherwise different from variety in any way

		Physical character.							Disease Suscept.					Insect Suscept.						
Entry	Rep	Plant vigor	Stunted growth/Plant ht.	Chloretic color	Flowering	Toasting (early, late)	Others?	# of Plants observed	% Plants affected (if any)	Leaf blight (describe)	Stalk rot (describe)	Ear rot (describe)	Others?	# of Plants observed	% Plants affected (if any)	Chloretic spp. (describe)	Necrotic spp. (describe)	Spider mites	Others?	# of Plants observed	% Plants affected (if any)	Comments/description
		X	X	X	X	X		1500		O	X	X		1500	1	X	X	X		1500		SOME NORTHERN LEAF BLIGHT (NORMAL)
		X	X	X	X	X		600		X	X	X	C	150	1	X	X	X		150		SOME MAIZE STRIPE VIRUS (NORMAL)

Date: 1-28-94 Trial #: 93-144-02N Cooperator: PIONEER HI-BRED PUERTO RICO INC.

Monitoring for Volunteer Plants

- After harvest and all data is collected, destroy unwanted vegetative material/ seed/tubers by the method(s) specified in the permit.
- If appropriate for your crop and area, irrigate after harvest to encourage germination.
- One month later, make the initial observation for the number of volunteers (estimates will suffice if the numbers are substantial). During the rest of the offseason, monitor on a monthly basis whenever the weather conditions are favorable for germination. Continue to monitor on a monthly basis for the period specified in the permit or until another transgenic test is planted in the same area.
- Record observations below.
- Remove any volunteer plants by hand weeding, herbicide treatments, or mechanical cultivation.
- Return form to Monsanto 12 months after harvest.

Number of volunteers observed -0-

Method used to destroy volunteers —

Comments Field was planted to oats - no volunteers were found under the oats canopy.

Ryan F. Baden — 9/16/94

Individual reporting observations — Date

Winterset Iowa — Study Title

Study Location

Release Permit Number 93-021-03
93-015

94-034-04 94-034-03

MONITORING FOR VOLUNTEER CORN PLANTS

Cooperator: Hawaiian Research

Monsanto Ref. No. 94-026-04N, 05N, 06N
94-023xRA

City and State: Kaiwa Ka Kai, Hawaii 96768

1. After harvest and all data is collected, unwanted vegetative material should be chopped or disked and plowed into the field. Harvested seed not returned to Monsanto or its cooperators may be rendered nonviable prior to its return to the plot area. Shelled cobs should be returned to the plot area or destroyed prior to disposal by burning, grinding or similar process.

2. If appropriate for your crop and area, irrigate after harvest to encourage germination. In Hawaii, see specific Department of Agriculture recommendation for a 30 day post-harvest fallow period with concurrent overhead irrigation.

3. One month after site tillage, make the initial observation for the number of volunteers (estimates will suffice if the numbers are substantial). Area of inspection should include both the transgenic plot area as well as accompanying 200 meter isolation distance if used. During the rest of the off-season, monitor on a monthly basis whenever the weather conditions are favorable for germination.

   Continue to monitor on a monthly basis until the end of the following growing season for corn in your area or until another transgenic test is planted in the same area. The plot area may not be planted with non-transgenic corn in the following season but may be planted to other appropriate rotational crops.

4. Record dates and observations below.

5. Remove any volunteer corn plants by handweeding, herbicide treatments, or mechanical cultivation.

6. Return form to Monsanto when monitoring is complete as defined above or 12 months after harvest, whichever is the lesser time period.

Date of Harvest: 7/8/94

Dates of Monitoring: 8/1/94 ; 8/31/94 ; 9/22/94

Number of Volunteers Observed: 8/1 = 59 plts/39 yd ; 8/31 = 15 plts/39 yd ; 9/22 = 0 plts/39 yd

Method Used to Destroy Volunteers: Mechanical Cultivation - Discing

Comments: _____

~~BZ~~ March 1993 planting in Kekaha
Harvested ~~July~~ 27, 1993

Mons #
92-016

Monitoring for Volunteer Plants

• After harvest and all data is collected, destroy unwanted seed/tubers by the method(s) specified in the permit.

• If available and appropriate for your crop and area, irrigate after harvest to encourage germination.

• One month later, make the initial observation for the number of volunteers (estimates will suffice if the numbers are substantial). During the rest of the offseason, monitor on a monthly basis whenever the weather conditions are favorable for germination. Continue to monitor on a monthly basis for the fallow period specified in the permit or until another transgenic test is planted in the same area.

• Record observations below.

• Remove any volunteer plants by hand weeding, herbicide treatments, or mechanical cultivation.

• Return form to Monsanto after every observation is complete.

Number of volunteers observed currently 0 Zero!

Method used to destroy volunteers cultivation with disc

Comments Harvest -> Burn -> Irrigate -> 100's of corn plants -> Disc -> Irrigate -> approx 20 corn plants -> Disc - Irrigate -> 0 corn plants

Rusty Smith
Individual reporting observations

9/18/93
Date

Kekaha
Study Location

RI Corn
Study Title

92-209-02

CBI DELETED 93-152

MONITORING FOR VOLUNTEER CORN PLANTS

Cooperator: [CBI DELETED] Monsanto Ref. No. 93306-04N BE+RRC

City and State: Isabela P.R.

1. After harvest and all data is collected, destroy unwanted vegetative material and or seed by methods specified in the EUP Protocol for the trial or in the Performance Standards in the front of the Compliance Notebook.

2. One month after site tillage, make the initial observation for the number of volunteers (estimates will suffice if the numbers are substantial). During the rest of the off-season, monitor on a monthly basis whenever the weather conditions are favorable for germination.

   Continue to monitor on a monthly basis into the following season for a total of an eight month period from trial harvest until the end of the following growing season for corn in your area . The plot area may not be planted with non-transgenic corn in the following season but may be planted to other appropriate rotational crops.

3. In Hawaii, Puerto Rico and Florida, follow harvest of the plot area with a 30 to 60 day fallow period during which the field should be irrigated and monitored for germinating corn plants. Record and destroy volunteer plants.

4. Record dates and observations below.

5. Remove any volunteer corn plants by handweeding, herbicide treatments, or mechanical cultivation.

6. Return form to Monsanto when monitoring is complete as defined above.

Date of Harvest: May 20 - May 24 (2nd); Feb. 1 - 4 (1st) [redacted]

Dates of Monitoring: monthly, during first week of month

Number of Volunteers Observed: 0-10/month

Method Used to Destroy Volunteers: Field Cultivation

Comments: NONE

RIHNJ702
RIHHVJ202
93-245-02N

4-month

MONITORING FOR VOLUNTEER CORN PLANTS

Cooperator: Pioneer Monsanto Ref. No. 93-099RA

City and State: Kekaha, HI

1. After harvest and all data is collected, unwanted vegetative material should be chopped or disked and plowed into the field. Harvested seed not returned to Monsanto or its cooperators may be rendered nonviable prior to its return to the plot area. Shelled cobs should be returned to the plot area or destroyed prior to disposal by burning, grinding or similar process.

2. If appropriate for your crop and area, irrigate after harvest to encourage germination. In Hawaii, see specific Department of Agriculture recommendation for a 30 day post-harvest fallow period with concurrent overhead irrigation.

3. One month after site tillage, make the initial observation for the number of volunteers (estimates will suffice if the numbers are substantial). Area of inspection should include both the transgenic plot area as well as accompanying 200 meter isolation distance if used. During the rest of the off-season, monitor on a monthly basis whenever the weather conditions are favorable for germination.

   Continue to monitor on a monthly basis until the end of the following growing season for corn in your area or until another transgenic test is planted in the same area. The plot area may not be planted with non-transgenic corn in the following season but may be planted to other appropriate rotational crops.

4. Record dates and observations below.

5. Remove any volunteer corn plants by handweeding, herbicide treatments, or mechanical cultivation.

6. Return form to Monsanto when monitoring is complete as defined above or 12 months after harvest, whichever is the lesser time period.

Date of Harvest: 2-23-94

Dates of Monitoring: 8-5-94

Number of Volunteers Observed: 0

Method Used to Destroy Volunteers: Irrigation & Disking etc.

Comments: Dates of Disking: 3-24, 4-12, 4-19, 5-3, 5-13, 5-29, 6-21, 8-1 1994

APPENDIX IV

MANAGEMENT OF INSECT PESTS WITH INSECT PROTECTED PLANTS: RECOMMENDED APPROACHES

Prepared by:

Monsanto Agricultural Group
St. Louis, MO

MANAGEMENT OF INSECT PESTS WITH INSECT PROTECTED PLANTS: RECOMMENDED APPROACHES

Monsanto Agricultural Group
St. Louis, MO

Abstract

Insect protected corn, cotton and potatoes, which exhibit a high level of protection to damage and yield loss by lepidopteran pests (corn and cotton) and the Colorado potato beetle (potatoes) have been developed through the expression of *B.t.* genes in plants. Monsanto has developed recommended approaches to utilize these plants to maximize the utility and durability of these new insect control products. These approaches are being tested and will be optimized in the field prior to commercial introduction of insect protected crops.

Introduction

Insect protected crops represent an important new management tool to control crop damage and loss due to insect pests. These plants offer significant benefits to the grower, the consumer and the environment. Insect resistance has been developed through the expression of genes that produce insecticidal proteins from *Bacillus thuringiensis* (*B.t.*) in the cells of the plants. The particular genes being developed by Monsanto for corn and cotton are derived from the *B.t.* subsp. *kurstaki* strain, and for potatoes from *B.t.* subsp. *tenebrionis*. These proteins are the basis of several commercially available microbial insecticides, which have been demonstrated as highly selective for insects, with no activity against other types of living organisms such as mammals, fish, birds or non-insect invertebrates (earthworms, spiders, etc.) (EPA, 1991; EPA, 1988). In addition, these proteins show a remarkable insect specificity (MacIntosh *et al.*, 1990). The *B.t.* genes developed for corn and cotton produce proteins that are active only against certain lepidopteran larvae with no activity against other orders of insects. Importantly, this activity spectrum overlaps with several important pests of

these crops which include the tobacco budworm, cotton bollworm or corn earworm, European corn borer, pink bollworm and several others such as cabbage looper, salt marsh caterpillar and cotton leaf perforator. Likewise, the *B.t.t.* gene developed for potatoes produces a protein active only against the Colorado potato beetle (CPB). Because these control agents are proteins, they have been found to break down rapidly in the environment and in mammalian digestive systems (Monsanto, 1993; Monsanto, 1994).

The use of insect protected plants will provide important benefits to growers, society and the environment (McGaughey and Whalon, 1992; Gasser and Fraley, 1989; Gould, 1988). First and foremost, these plants offer an alternative to chemical insecticides currently used to control susceptible insect pests with efficacy equal to or better than that of current control methods. The use of insect protected corn, cotton and potatoes will significantly reduce the application of chemical insecticides directed at these pests. The reduction of insecticide use will have direct benefits to the grower, such as less time and effort spent on insect control and reduced exposure to chemical insecticides.

Insect protected crops are also likely to produce secondary benefits in pest control as an indirect result of the reduction in use of chemical insecticides. Chemical insecticides like pyrethroids are relatively non-specific and have the effect of killing beneficial predatory and parasitic insects (Roush and Tingey, 1993; Van den Bosch and Stern, 1962). Because the *B.t.* proteins produced by insect protected plants are not active against these beneficial insects, populations have been shown to rise significantly in fields planted with insect protected cotton and CPB protected potatoes compared to nontransgenic cotton and potatoes treated with chemical insecticides (Feldman, *et al.*, 1993; Reed *et al.*, 1992; Stone *et al.*, 1992; Luttrell, pers. comm.). Preserving the beneficial insect population should enhance the biological control of both target pests and non-target pests such as mites, aphids, and leafhoppers, which increase as problems as their natural predators are removed. In addition, insect protected corn and cotton and CPB protected potatoes are equally capable of controlling target pest populations, which are beginning to lose their sensitivity to chemical insecticides (Everich, 1994; Stone and Sims, 1993), thus filling a need that is likely to grow in coming years.

The use of insect protected plants will provide important benefits to growers, society and the environment. To achieve these benefits, it is important that insect protected plant strategies be implemented and managed properly. In this respect, these plants are no different than any other pesticide. There are two aspects of this management. First, is the development of pest management techniques that allow the farmer to maximize the ability of these plants to control target pests. In essence, this is the development of a total insect management package that will be centered around a new tool, insect protected corn, cotton or potatoes. Second, is the development of appropriate strategies to maximize the product durability and the utility of insect protected crops. Part of this management program is the development and implementation of strategies targeted to prevent the development of insect resistance to the *B.t.* proteins produced by these plants. Because both management aspects can affect the way in which insect protected plants are used by the grower, these two types of management, total pest management and insect resistance management, are interconnected.

Resistance management is not an issue particular to insect protected plants, given the development of insect resistance to chemical insecticides. Monsanto scientists have addressed insect resistance for several years in laboratory and field studies and with outside collaborators we have examined nearly every suggestion that has been made for resistance management in insect protected plants (Everich, 1994; Roush, 1994; Sachs, 1993; Stone and Sims, 1993). As the following discussion will demonstrate, promising strategies for resistance management for insect protected plants are available and can be recommended. These strategies have been developed in consultation with an expert advisory panel established for each crop taking into account existing research and an understanding of crop production and agronomic practices. Consequently, these strategies may be specific for each crop and target pest. It is evident, however, that insect protected plants offer some unique options in pest and resistance management that are not available with traditional pesticides.

Integrated Pest and Resistance Management with Insect Protected Plants

As part of a package to provide economic control of insect loss and damage in corn, cotton and potatoes, these insect protected crops will provide a central focus around which other insect management practices will be applied. In

many areas lepidopteran pests are the primary damaging insects of corn and cotton, so the use of these insect protected plants to control these pests will be a major portion of total insect control. The primary pest in potato production is the CPB. Its control impacts the populations of other pests such as aphids and leafhoppers. By substituting genetically modified corn, cotton or potatoes for chemical pesticides directed at their target pests, a positive impact on overall insect management will result. Many of the details of pest management with insect protected plants can only be determined by multi-year large scale field tests designed to incorporate these genetically modified crops into current production practices. Such field trials are in progress and are providing the data needed for developing a pest and resistance management program for these crops. These trials involve collaborations between Monsanto, NatureMark™ (a wholly owned subsidiary of Monsanto), seed company partners, and academic and extension entomologists. They are examining the impact of insect protected plants on populations of beneficial and pest insects endemic to the crops and the impact on the use of conventional insecticides for controlling non-target pests (Feldman, *et al.*, 1993; Reed *et al.*, 1992; Stone *et al.*, 1992), the establishment of the baseline susceptibility of our insect targets to *B.t.* protein (Stone and Sims, 1993; Everich, 1994; Luttrell, pers. comm.) and the impact of mixtures of protected and non-protected plants on yield loss (Roush, 1994).

Insect protected corn, cotton and CPB protected potatoes will be important additions to the available methods of controlling insect pests. The implementation of these plants is fully consistent with the goals of integrated pest management because:

a) the *B.t.* protein produced by the plants is insect specific, affecting only a few targeted pest species

b) the *B.t.* protein is active only against insects feeding on the plant and thus doing damage

c) use of the plants will reduce the application of chemical insecticides

d) use of the plants will preserve beneficial insects, which will enhance the biological control of non-target pests

Because pest and resistance management are interconnected, it is important to develop both of these approaches in tandem for each insect protected crop.

Combination of Insect Protected Plants with Chemical Insecticides

One aspect of the use of insect protected plants for integrated pest management in corn, cotton and potatoes is the continued use of chemical insecticides. Some insecticides will continue to be used in these crops for non-target pests. If possible, these insecticides need to be chosen so as to not negatively impact beneficial arthropods, which are integral in the biological control of non-susceptible species. The combination of insect protected crops with chemical insecticides, while part of a total insect control package, is not a resistance management option for insect protected plants per se. Chemical insecticides can reduce the population size of insects selected for resistance to *B.t.* but cannot alter the gene frequencies within this population (Roush, 1989). Alternatively, insect protected plants should positively impact current chemical insecticides by helping slow resistance development and prolonging the life of these important agricultural chemicals.

Resistance Management for Insect Protected Plants

As described above, part of managing the implementation of insect protected plants is the design and implementation of appropriate strategies to delay or prevent the development of insect resistance to *B.t.* protein in corn, cotton or potatoes. Described below are approaches that will help manage resistance development in these crops. It is important to note that: 1) as insect resistance development is a biological phenomenon, the rate of development is difficult if not impossible to predict and consequently, the efficacy of a strategy to delay or prevent its development may be impossible to demonstrate; 2) because of the available technology, biology of the pest, and the production practices of the crop, implementation of these strategies will be dependent on the crop and the target pest; and 3) field research must be conducted to determine the practical implementation of these strategies within current crop production practices. These strategies have been recommended by several researchers (Gould, 1988; Stone *et al.*, 1991; McGaughey and Whalon, 1992) and are summarized briefly below and then expanded in greater detail in the next section.

Summary of Considered Resistance Management Strategies for Insect Protected Corn, Cotton and Potatoes

- High dose expression of *B.t.* protein in plants to control insects heterozygous for resistance alleles.
- Refugia as hosts for sensitive insects provided through non-insect protected plants or other non-modified hosts.
- Monitoring of insect populations for susceptibility to *B.t.* protein.
- Agronomic practices that minimize insect exposure to *B.t.* plants.
- Integrated pest management (as described above).
- Combination of multiple genes within the same corn plant, both of which are active against targeted insects but with different sites/modes of action.
- Incorporation of host plant resistance traits into insect protected corn and cotton as they are proven effective.
- Incorporation of novel proteins that provide effective control of targeted pests.

Details of Resistance Management Strategies

High Dose Expression

High dose expression for resistance management is based on three assumptions:

1) Resistance will most likely be controlled by one major locus with recessive resistance alleles (McGaughey and Beeman, 1988; MacIntosh *et al.*, 1991; Sims and Stone, 1991).

2) Insects developing resistance to the *B.t.* protein will be rare initially and will almost always mate with susceptible insects giving rise to heterozygous progeny (Gould, 1986).

This type of refuge will exist in all the acres not covered by these insect protected plants. This area will be substantial in the early years after introduction and could supply a sufficient refuge for several years. As insect protected seed becomes more available and widely grown, this refuge will be reduced. Consequently, over time, reliance on non-insect protected corn, cotton or potato fields for refugia may not be adequate.

2) Refuge outside of the crop: Non-modified crop hosts.

The European corn borer and the cotton bollworm or corn earworm have many non-corn or cotton hosts including other crops in all locations, which may provide an adequate refuge. The tobacco budworm and Colorado potato beetle have fewer alternatives and the pink bollworm has none. In some locations corn, cotton and potatoes may be the only host for at least one insect generation per season. The use of *B.t.* microbials or transgenic *B.t.* plants on other crops will also impact their utility as a refuge for insect protected plants. This option must be evaluated carefully based on the crop, pest biology, and growing regions.

3) Refuge within the crop: Non-insect protected plants.

In certain cases a likely solution is to provide an "in crop" refuge of non-insect protected plants. For this in crop refuge, the choices are: a) random mixture of seed of insect protected and non-protected plants or b) non-insect protected plants planted within the same field. The optimum refuge area required must be determined for each crop.

Mixed seed lines (*B.t.* and non-insect protected seed within the same bag) have a certain appeal due to the "automatic" implementation. A possible problem with mixed seed arises from larvae that survive on a non-insect protected plant and migrate to a modified plant where they may be less sensitive to *B.t.* protein because of their size. This could compromise insect control and increase selection pressure for resistance. The likelihood of this occurring is being investigated experimentally before this strategy is implemented.

There may also be economic and logistical problems if a mixed seed strategy is implemented. However, Monsanto, NatureMark™ and seed company partners are interested in determining the viability of the mixed seed approach. It is clear that field research is required to determine the

Pyramiding Traits

A set of strategies for the medium and long term focus on combining multiple insecticidal agents. The rationale is essentially the same for all of these: Expose the insects to two or more active agents with distinct modes of action at the same time, and the probability of any one insect being selected for resistance to both agents simultaneously is extremely low.

1) Combination with a Second Insect Resistance Gene

A second gene within the same plant possessing a different mode of action will significantly reduce the frequency of resistant individuals (Peferoen, 1992; Stone *et al.*, 1991; Van Rie, 1991). Population models indicate that other alternative uses of a second gene such as seed mixture or using single genes in rotation, may be as effective as two genes within the same plant (Gould, 1988; Gould 1986). Assuming initial gene frequencies for *B.t.* protein resistance are low, initial introduction of a product with a single *B.t.* gene should not negatively compromise a second gene because the single gene product will be planted on limited acres in the first few years. In the medium term the best choice of second gene is an unrelated *B.t.* gene. In the long term, the use of novel, non-*B.t.* insecticidal genes holds great promise. This area is under active research.

2) Combination with Host Plant Resistance Traits

This is a long term strategy to be implemented by seed companies or public breeders. Host plant resistance traits (HPR) used in combination with insect protected corn and cotton need to be insecticidally effective and not negatively impact quality or yield. For example, Monsanto currently has funded research on HPR to help set direction on HPR traits that alone or in combination are useful in protecting the plant from lepidopteran insects in cotton (Sachs, 1993). Cotton seed companies are interested in incorporating these traits if they are effective and have no negative effects on yield or quality. Similar work is planned with insect protected corn.

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Notices

Federal Register
Vol. 61, No. 52
Friday, March 15, 1996

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

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 96-006-1]

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

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

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

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

SUPPLEMENTARY INFORMATION: On September 5, 1995, the Animal and Plant Health Inspection Service (APHIS) published a notice in the Federal Register (60 FR 46107-46108, Docket No. 95-041-2) announcing the issuance of a determination effective August 22, 1995, that an insect resistant corn line developed by the Monsanto Company (Monsanto) designated as corn line MON 80100, does not present a plant pest risk and is not a regulated article under the regulations contained in 7 CFR part 340. This action was in response to a petition submitted by Monsanto seeking a determination from APHIS that its corn line MON 80100 no longer be deemed a regulated article, based on an absence of plant pest risk. The effect of that action was that the subject corn line and its progeny would no longer be regulated under the regulations in 7 CFR part 340.

The two additional corn lines that are the subject of this notice, MON 809 and MON 810, were identified in Monsanto's previously submitted petition (APHIS Petition No. 95-093-01p) for corn line MON 80100. On January 17, 1996, APHIS received additional information and field test data in a petition (APHIS Petition No. 96-017-01p) in support of nonregulated status under 7 CFR part 340 for corn lines MON 809 and MON 810. As described by Monsanto, corn lines MON 809 and MON 810 express a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* which confers resistance to European corn borer. The subject corn lines were generated through use of the particle acceleration transformation system to insert plasmid vectors PV-ZMBK07 and PV-ZMCT10, the same vectors used to transform corn line MON 80100 for which the August 22, 1995, determination of nonregulated status was issued by APHIS.

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

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

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96-6201 Filed 3-14-96; 8:45 am]

BILLING CODE 3410-34-P

Monsanto

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Phone (314) 694-1000

February 13, 1995

Dr. Vedpal Malik
Biotechnology Permit Unit
Biotechnology, Biologics and Environmental Protection
USDA-APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Dear Dr. Malik:

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

Sincerely,

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

Molecular Analysis of YieldGard™ Corn Line MON 809

Janice Kania, Pamela Keck and Patricia Sanders

I. SUMMARY

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

Summary of Corn Line MON 809 Molecular Analysis

Genetic Element	23 Kb insert
cryIA(b) gene	1 full length, 1 partial
CP4 EPSPS gene	2 full length
gox gene	1 partial
nptII / ori-pUC	present

II. RESULTS AND DISCUSSION

A. Southern blot results

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

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

B. Insert Number

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

C. Insert Composition

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

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

corn line MON 809 (data not shown). The NcoI/EcoRI digests, probed with the CP4 EPSPS gene, identified only the expected size CP4 EPSPS gene.

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

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

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

III. CONCLUSIONS

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

Based on these analyses, we conclude that corn line MON 809 contains a single I-DNA with an intact *cryIA(b)* gene and two CP4 EPSPS genes that are responsible for producing the correct size CryIA(b) and CP4 EPSPS proteins.

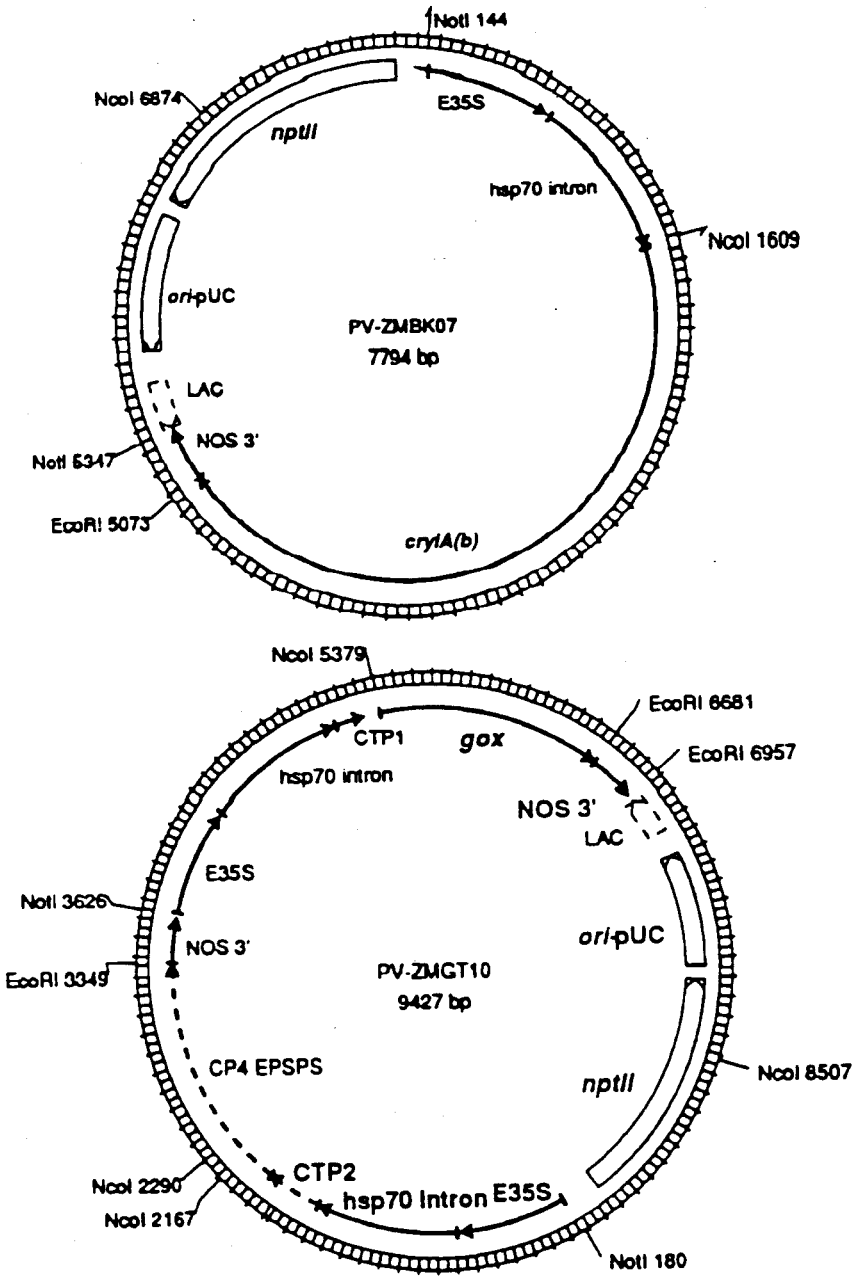

Figure 1. Plasmid maps of PV-ZMBK07 and PV-ZMGT10. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.

Figure 2. Southern blot analysis of corn line MON 809 DNA: insert number analysis

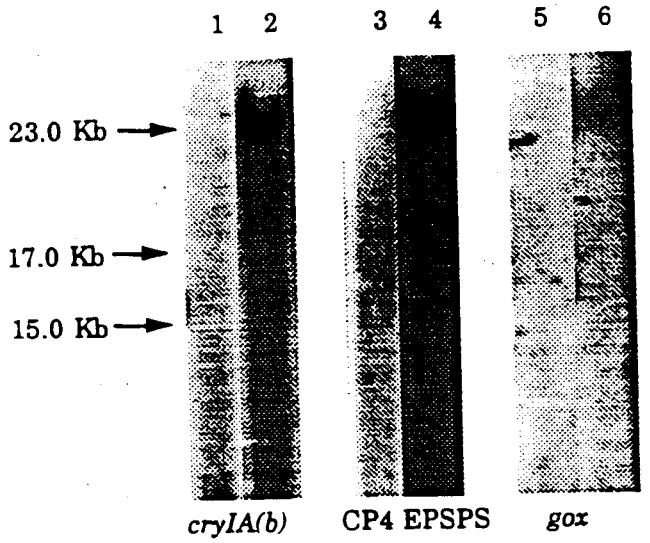

Figure 2. Southern blot analysis of corn line MON 809 DNA. Lanes 1, 3 and 5 contain 12.5 µg of corn line MON 818 DNA digested with NdeI. Lanes 2, 4 and 6 contain 12.5 µg of corn line MON 809 DNA digested with NdeI. Lanes 1 and 2 were hybridized with the *cryIA(b)* gene. Lanes 3 and 4 were hybridized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

→ Symbol denotes sizes obtained from MW markers.

Figure 3. Southern blot analysis of corn line MON 809 DNA: *cryIA(b)* gene analysis

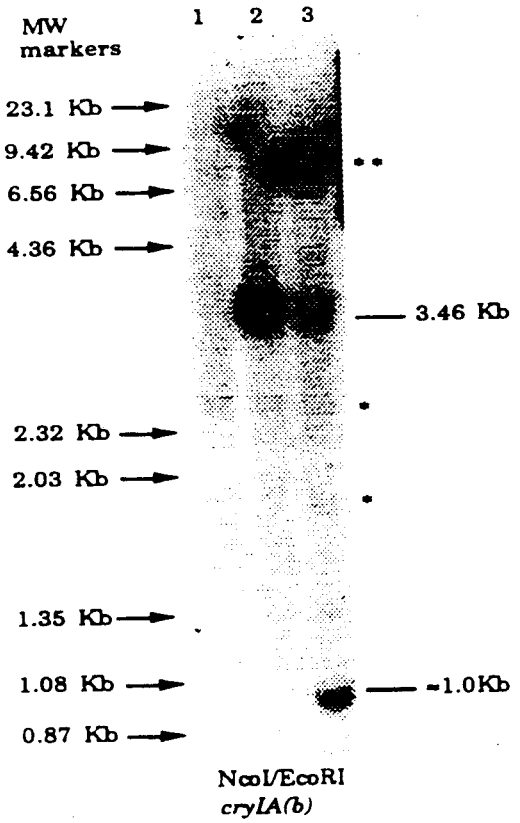

Figure 3. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *cryIA(b)* gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.
* Symbol denotes background bands (≈2.5 and 1.9 Kb).
~ Symbol denotes a band size approximated from MW marker and plasmid digests.
* * Symbol denotes an area of non-specific hybridization. This is supported by the observation that the signal is between two lanes.

Figure 4. Southern blot analysis of corn line MON 809 DNA: CP4 EPSPS gene analysis

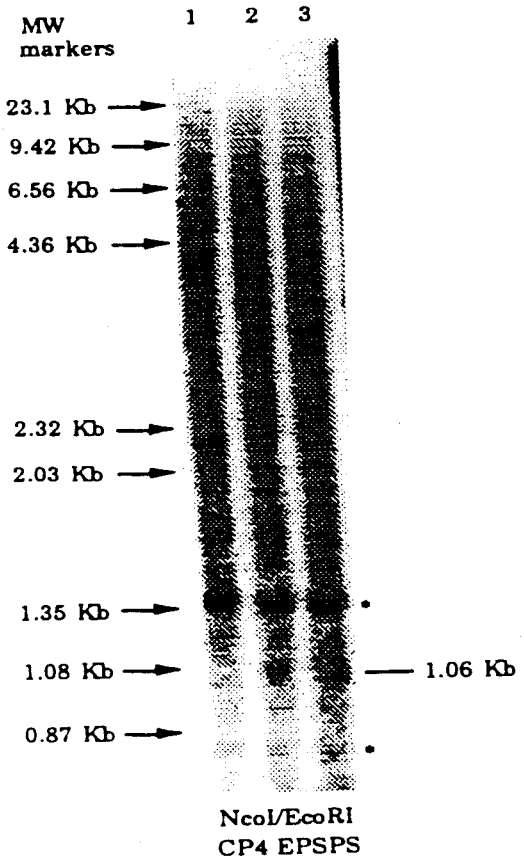

Figure 4. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the CP4 EPSPS gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.
* Symbol denotes background bands (≈1.37 and 0.80 Kb).
~ Symbol denotes a band size approximated from MW marker and plasmid digests.

Figure 5. Southern blot analysis of corn line MON 809 DNA: *gox* gene analysis

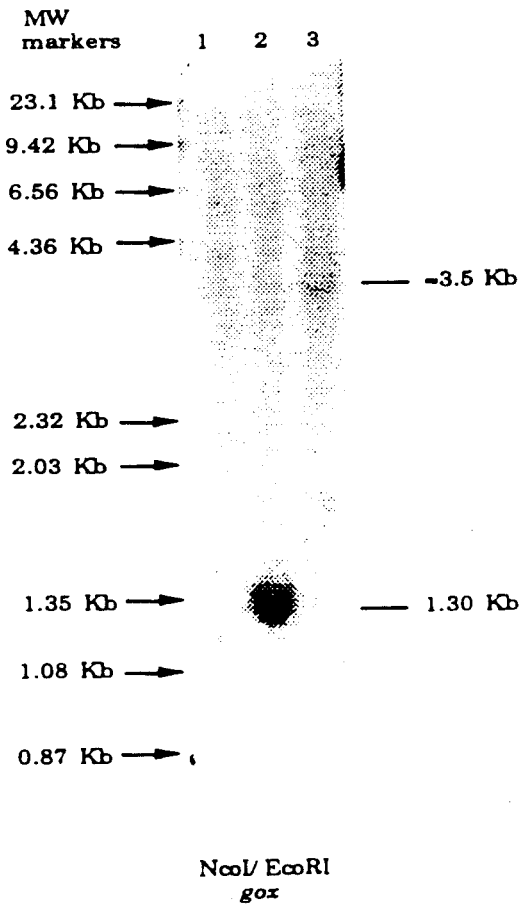

Figure 5. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *gox* gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.
≈ Symbol denotes a band size approximated from MW marker and plasmid digests.

Figure 6. Southern blot analysis of corn line MON 809 DNA: *nptII* and *ori-p*UC analysis

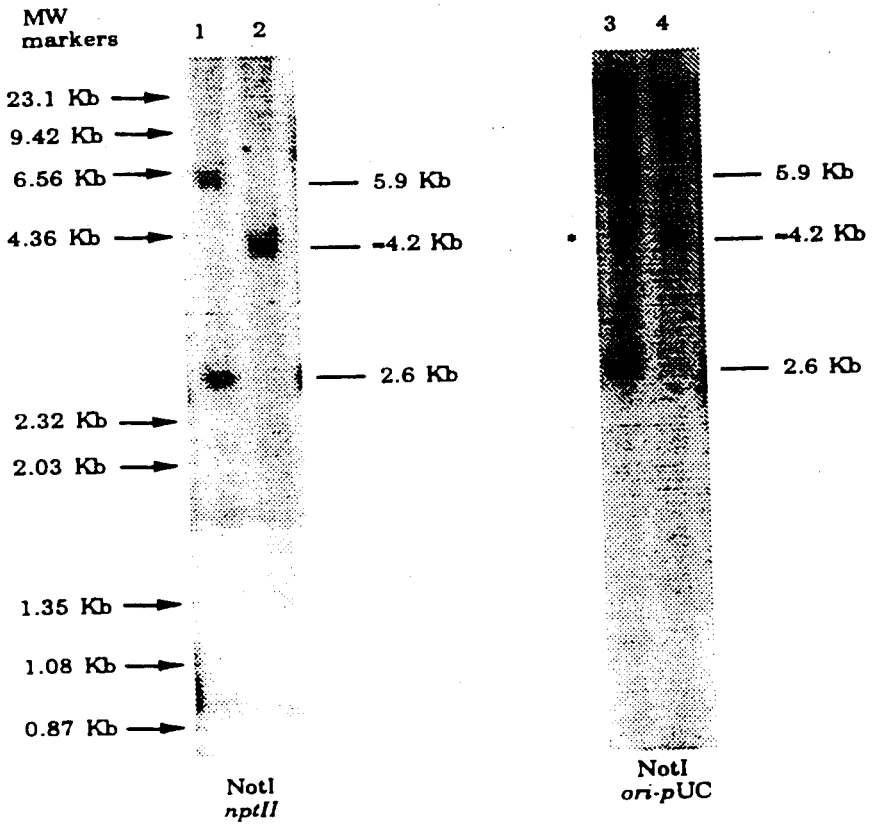

Figure 6. Southern blot analysis of corn line MON 809 DNA. Lanes 1-4 contain the following DNAs digested with NotI: lanes 1 and 3, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lanes 2 and 4, MON 809 DNA. Lanes 1 and 2 were hybridized with the *nptII* region. Lanes 3 and 4 were hybridized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

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

Molecular Analysis of Insect Protected Maize Line MON 810

Janice Kania, Pamela Keck, Elaine Levine and Patricia Sanders

I. SUMMARY

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

Genetic Element	Maize Line MON 810
cryIA(b) gene	present
CP4 EPSPS gene	not present
gox gene	not present
nptII/*ori*-pUC	not present

II. RESULTS AND DISCUSSION

A. Southern blot results

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

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

B. Insert Number

NdeI digestion results. The purpose of the NdeI digests was to determine the number of plasmid DNA inserts in the maize line MON 810. The plasmids PV-ZMBK07 and PV-ZMGT10 do not contain a restriction site for NdeI. Thus this enzyme effectively cleaves outside any inserted DNA, releasing a fragment containing the inserted DNA and adjacent genomic DNA. MON 818 control DNA and MON 810 DNA were digested with NdeI and probed with plasmid PV-ZMBK07 DNA. The results are shown in Figure 2. MON 818 DNA (lane 1), produced one very light, diffused band of approximately 21.0 Kb which is a background band since it is present in both the control MON 818 DNA and the MON 810 DNA. MON 810 DNA produced one band, approximately 5.5 Kb in size (lane 2). This result established that insect protected maize line MON 810 contains one fragment of integrated DNA. The size of the inserted DNA plus adjacent genomic DNA up to the NdeI restriction sites is approximately 5.5 Kb.

C. Insert Composition

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

2. CP4 EPSPS gene integrity. Plasmid DNAs (PV-ZMBK07 and PV-ZMGT10) and insect protected maize line MON 810 DNA were digested with NcoI/BamHI to release the CP4 EPSPS gene and the Southern blot probed with the CP4 EPSPS gene. The results are shown in Figure 4, lanes 1 and 2. Approximately 50 pg of a mixture of PV-ZMBK07 and PV-ZMGT10 DNA (lane 1) produced one band, approximately 3.1 Kb in size, which corresponds to the expected size CP4 EPSPS fragment, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). MON 810 DNA (lane 2) shows no hybridizing fragements to the CP4 EPSPS probe, establishing that insect protected maize line MON 810 does not contain the CP4 EPSPS gene.

3. *gox* gene integrity. Plasmid DNAs (PV-ZMBK07 and PV-ZMGT10) and insect protected maize line MON 810 DNA were digested with NcoI/BamHI to release the *gox* gene and the Southern blot probed with the *gox* gene. The results are shown in Figure 4, lanes 3 and 4. Approximately 50 pg of a mixture of PV-ZMBK07 and PV-ZMGT10 DNA (lane 3) produced one band, a NcoI/NcoI fragment, approximately 3.1 Kb, which corresponds to the expected size *gox* fragment, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). MON

810 DNA (lane 4) shows no hybridizing fragments to the *gox* probe, establishing that insect protected maize line MON 810 does not contain the *gox* gene.

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

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

V. CONCLUSIONS

The insect protected maize line MON 810 was produced by particle acceleration technology with a DNA solution that contained the *cryIA(b)*, CP4 EPSPS, *gox* and *nptII* genes. Maize line MON 810 contains one integrated DNA contained on a 5.5 Kb NdeI fragment, which contains the E35S promoter, maize hsp70 intron and the *cryIA(b)* gene. Insect protected maize line MON 810 does not contain a CP4 EPSPS gene, a *gox* gene or *nptII/ori-p*UC sequences. The continued efficacy of maize line MON 810 confirms that an insecticidally active CryIA(b) protein is produced which provides season long control of European Corn Borer.

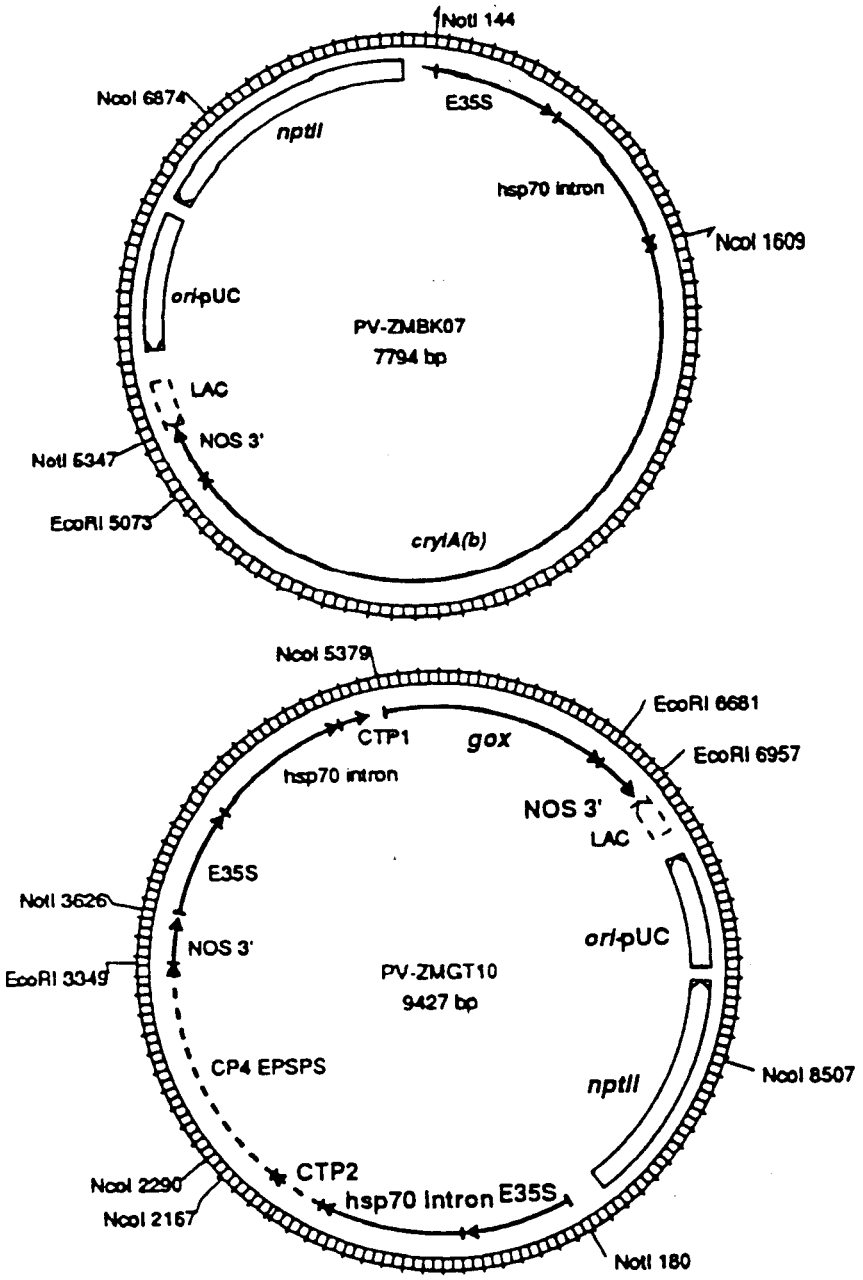

Figure 1. Plasmid maps of PV-ZMBK07 and PV-ZMGT10. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.

Figure 2. Southern blot analysis of maize line MON 810 DNA: insert number analysis

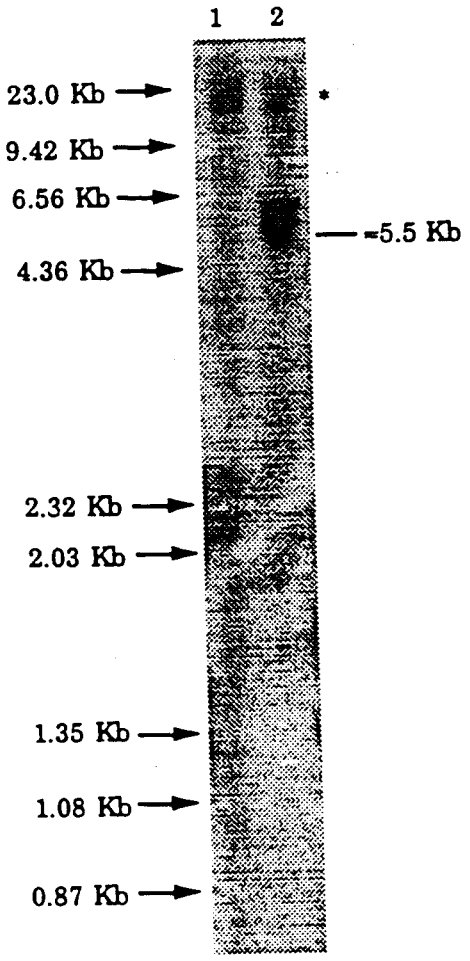

Figure 2. Southern blot analysis of maize line MON 810 DNA. Lanes 1 and 2 contain the following DNAs digested with NdeI and probed with PV-ZMBK07: lane 1, MON 818 DNA; lane 2, MON 810 DNA.

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

Figure 3. Southern blot analysis of maize line MON 810 DNA: *cryIA(b)* gene analysis

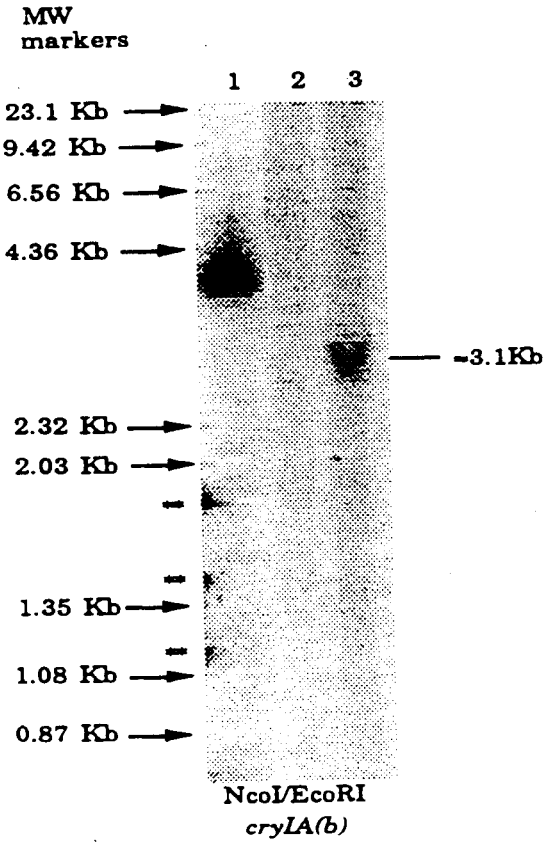

Figure 3. Southern blot analysis of maize line MON 810 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *cryIA(b)* gene: lane 1, ≈50 pg of plasmid PV-ZMBK07; lane 2, MON 818 DNA, lane 3, MON 810 DNA.

⟶	Symbol denotes sizes obtained from MW markers on ethidium stained gel.
—	Symbol denotes sizes obtained from plasmid digests.
-	Symbol denotes a band size approximated from MW marker and plasmid digests.
* *	Symbol denotes an area of hybridization in an adjacent lane which only appears to be in lane 1, due to the contents of the lanes migrating at an angle in this portion of the gel.

Figure 4. Southern blot analysis of maize line MON 810 DNA: CP4 EPSPS and *gox* gene analysis

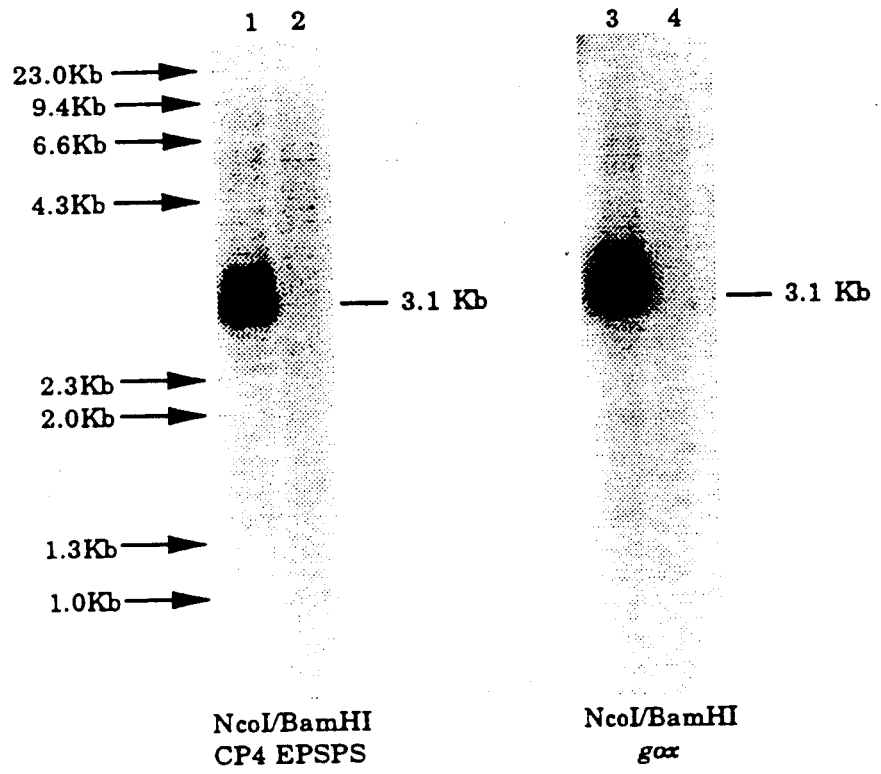

Figure 4. Southern blot analysis of maize line MON 810 DNA. Lanes 1-4 contain the following DNAs digested with NcoI/BamHI: lanes 1 and 3, ≈50pg of plasmids PV-ZMGT10 and PV-ZMBK07; lanes 2 and 4, MON 810 DNA . Lanes 1 and 2 were hybridized with the CP4 EPSPS gene. Lanes 3 and 4 were hybridized with the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

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.

Figure 5. Southern blot analysis of maize line MON 810 DNA: *nptII* and *ori-p*UC analysis

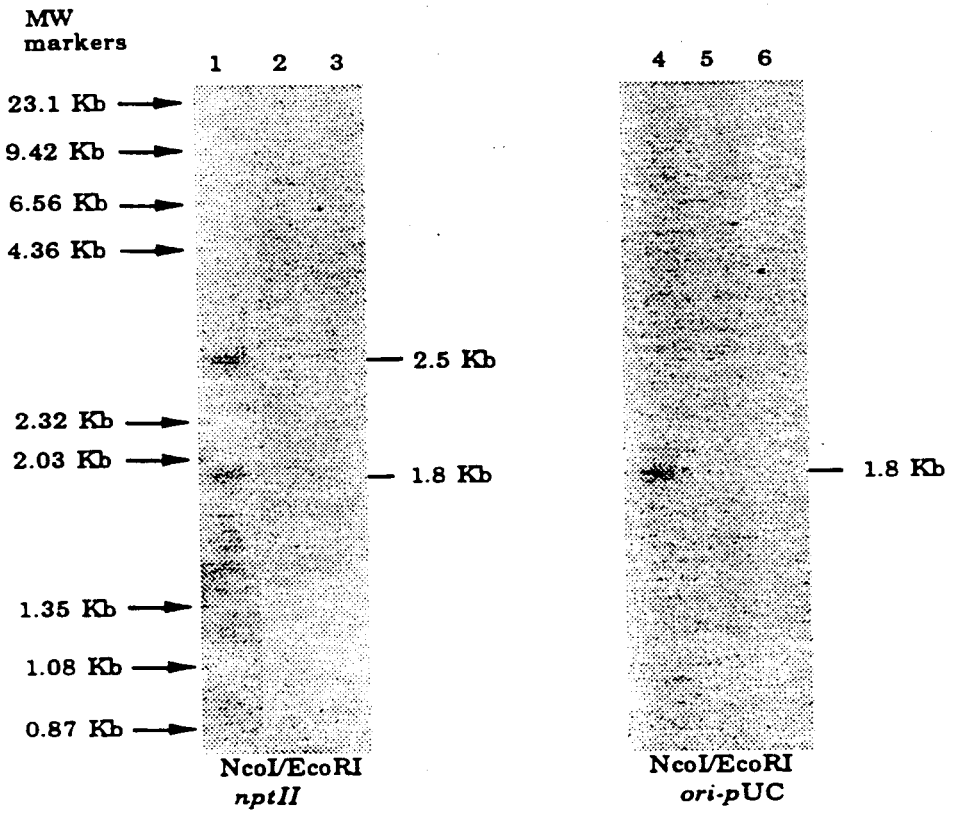

Figure 5. Southern blot analysis of maize line MON 810 DNA. Lanes 1-6 contain the following DNAs digested with NcoI/EcoRI: lanes 1 and 4, ≈50pg of plasmid PV-ZMBK07; lanes 2 and 5, MON 818 DNA; lanes 3 and 6, MON 810 DNA. Lanes 1-3 were hybridized with the *nptII* region. Lanes 4-6 were hybridized with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

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.

Monsanto

96-017-01P

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

January 8, 1996

Mr. Michael A. Lidsky
Deputy Director, BBEP, APHIS, USDA
6505 Belcrest Road
Federal Building
Hyattsville, MD 20782

Subject: Petition for Determination of Non-Regulated Status: Additional YieldGard™ Corn (*Zea mays* L.) Lines with the cryIA(b) Gene from *Bacillus thuringiensis* subsp. *kurstaki*.
Monsanto #: 95-274U

Dear Mr. Lidsky:

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

This petition requests a determination from APHIS that YieldGard™ corn lines MON 809 and 810, any progenies derived from crosses between MON 809 and 810 and traditional corn varieties, and any progeny derived from crosses of MON 809 and 810 with transgenic corn varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under regulations in 7 CFR part 340. These two additional corn lines were originally identified in USDA Petition 95-093-01p for YieldGard corn line MON 80100 submitted to the agency on March 30, 1995 and approved August 22, 1995 (FR 60:171; pp. 46107-46108).

1-11-96

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

Sincerely,

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

cc: Dr. C.T. Dickerson - Monsanto

Petition for Determination of Nonregulated Status:

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

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

Submitted by:

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

January 8, 1996
#95-274U

Prepared by:

K.A. Croon, P.R. Sanders, J. Kania, P. Keck, E. Levine, and G.B. Parker

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

Summary

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

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

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

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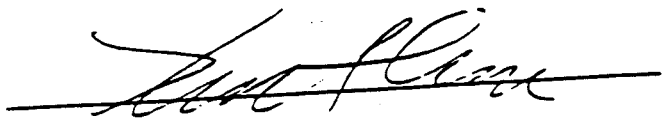
and is safe to non-target organisms such as fish, birds, mammals, and beneficial insects. In addition, the risk of an uncontrolled introduction of this corn into the environment through hybridization or outcrossing to native species is virtually non-existent in the U.S.

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

Therefore, Monsanto Company requests a determination from APHIS that YieldGard corn lines MON 809 and 810 and any progenies derived from crosses between MON 809 and 810 and traditional corn varieties no longer be considered regulated articles under regulations in 7 CFR part 340.

Certification

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner which are unfavorable to the petition.



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Abbreviations Used in this Petition for the Determination of Non-Regulated Status of Additional YieldGard Corn Lines MON 809 and 810

APHIS	Animal Plant Health Inspection Service
bp, Kb	Base pairs, kilobase pairs
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)	Gene for class I (Lepidoptera-specific) crystal protein
CryIA(b)	Class I (Lepidoptera-specific) crystal protein
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
FDA	Food and Drug Administration
FFDCA	Federal Food Drug and Cosmetic Act
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
GLP	Good Laboratory Practice
gox	Gene for glyphosate oxidase
GOX	Glyphosate oxidase protein
hsp70	Intron sequence from heat-shock protein 70
I-DNA	Integrated DNA
IPM	Integrated Pest Management
NOS 3'	3' transcriptional termination sequence from nopaline synthase
NPTII	Neomycin phosphotransferase II
nptII	Gene for neomycin phosphotransferase II
ori-pUC	Bacterial origin of replication from the pUC plasmid
sp	Species
subsp.	Subspecies
USDA	United States Department of Agriculture
µg, g	Microgram, gram

PETITION FOR DETERMINATION OF NON-REGULATED STATUS OF ADDITIONAL YIELDGARD CORN LINES MON 809 AND 810

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Part I. Rationale for Development of YieldGard™ Corn

A. Need and Benefits of Yieldgard Corn

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

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

Monsanto has developed genetically modified corn plants (YieldGard™) that control ECB. This YieldGard corn offers a new mechanism to produce and deliver a highly effective insecticide to target pests (e.g. production by cells of the crop plant rather than industrially and application by spray equipment). The technology couples the environmental advantages of host plant resistance with the efficacy of CryIA(b), an effective biological

insecticide. YieldGard corn expresses the CryIA(b) protein which is selective against certain lepidopteran insects that must feed upon the plants to be controlled. Therefore, this technology offers selective activity without disrupting pest suppression by natural enemies, such as parasites and predators.

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

- A more reliable, economical, and less labor intensive means to control ECB.
- Insect control without harming non-target species, including humans.
- A means for growers to significantly reduce the amount of chemical insecticides now applied to the crop thereby achieving ECB control in a more environmentally compatible manner than is currently available.
- A reduction in the manufacturing, shipment, and storage of chemical insecticides used in corn.
- A reduction in the exposure to workers to the pesticide and pesticide spray solution.
- A reduction in the number of empty pesticide containers and amount of pesticide spray solution that must be disposed of according to applicable environmental regulations.
- An ideal fit with Integrated Pest Management (IPM) and sustainable agricultural systems.
- Both large and small growers will benefit from the planting of YieldGard corn as no additional labor, planning, or machinery is required.

B. Regulatory Approvals

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

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

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

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

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

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

C. References

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Part II. The Corn Family

A. Summary

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

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

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

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Part III. Description of the Transformation System and Plasmids Utilized

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

YieldGard corn lines MON 809 and 810 were produced with a DNA solution containing two plasmid vectors, PV-ZMBK07 and PV-ZMGT10. Plasmid DNA was introduced into the plant tissue using the particle acceleration method as previously identified (USDA Petition 95-093-01p). The PV-ZMBK07 plasmid contains the *cryIA(b)* gene and PV-ZMGT10 contains the CP4 EPSPS and *gox* genes. Both plasmids contain the *nptII* gene under the control of a bacterial promoter and an origin of replication from a pUC plasmid, required for selection and replication in bacteria, respectively. The plasmid vector PV-ZMBK07 is shown in Figure III.1 and PV-ZMGT10 is shown in Figure III.2. A description of the DNA elements in PV-ZMBK07 and PV-ZMGT10 are provided in Tables III.1 and III.2, respectively.

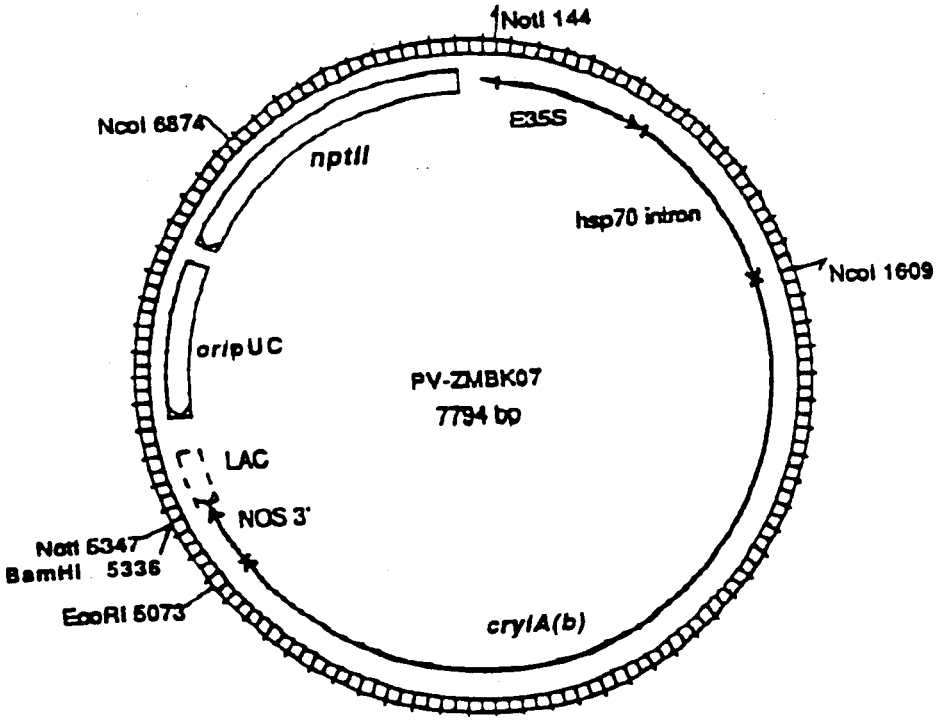

Figure III.1 Plasmid map of PV-ZMBK07.

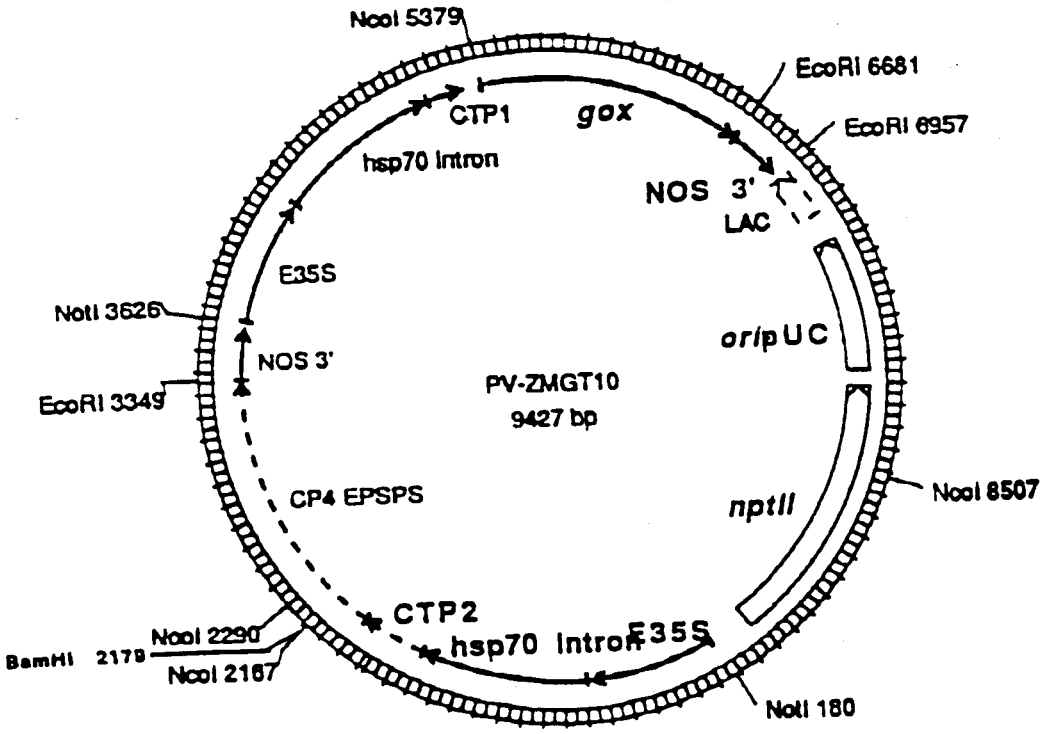

Figure III.2 Plasmid map of PV-ZMGT10.

Table III.1 Summary of DNA elements in the plasmid PV-ZMBK07

Genetic Element	Size, Kb	Function
E35S	0.61	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1985).
hsp70 intron	0.80	Intron from the maize *hsp70* gene (heat-shock protein) present to increase the level of gene transcription (Rochester *et al.*, 1986).
cryIA(b)	3.46	The gene encodes the nature identical CryIA(b) protein product (Fischhoff *et al.*, 1987).
NOS 3'	0.26	A 3' nontranslated region of the nopaline synthase gene which terminates transcription and directs polyadenylation (Fraley *et al.*, 1983).
lacZ	0.24	A partial *E. coli lacI* coding sequence, the promoter Plac, and a partial coding sequence for beta-D-galactosidase or *lacZ* protein from pUC119 (Yanisch-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	0.79	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).

Table III.2 Summary of DNA elements in the plasmid PV-ZMGT10

Genetic Element	Size, Kb	Function
E35S	0.61	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1985).
hsp70 intron	0.80	Intron from the maize *hsp70* gene (heat-shock protein) present to increase the level of gene transcription (Rochester *et al.*, 1986).
CTP2	0.31	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS (Klee and Rogers, 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) which allows for the selection of transformed cells on glyphosate.
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).
NOS 3'	0.26	A 3' nontranslated region of the nopaline synthase gene which terminates transcription and directs polyadenylation (Fraley *et al.*, 1983).
lacZ	0.24	A partial *E. coli lacI* coding sequence, the promoter Plac, and a partial coding sequence for beta-D-galactosidase or *lacZ* protein from pUC119 (Yanisch-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	0.79	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).

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Part IV. Molecular Biology of YieldGard Corn Lines MON 809 and 810

Introduction

As described in USDA Petition 95-093-01p, YieldGard corn lines MON 809 and 810 were generated using a particle acceleration transformation system with a DNA solution containing the two plasmid vectors, PV-ZMBK07 and PV-ZMGT10. The *cryIA(b)* gene was inserted to confer resistance to certain lepidopteran insects while the CP4 EPSPS and *gox* genes produce proteins which confer tolerance to glyphosate, a selective agent used to identify plant cells expressing the *cryIA(b)* gene. In addition to these three genes, a *nptII* gene which produces the enzyme neomycin phosphotransferase II (NPTII) was present in the two vectors under the control of its own bacterial promoter, to enable selection in bacterial systems.

A. Molecular Analysis of YieldGard Corn Line MON 809

This summary describes the molecular analysis of the integrated DNA (I-DNA) present in YieldGard corn line MON 809. Specifically, the insert number (number of integration sites within the corn genome) and the number and integrity of each inserted gene were determined. Corn line MON 809 was produced by particle acceleration technology using a DNA solution containing two plasmids, PV-ZMBK07 [*cryIA(b)* gene]and PV-ZMGT10 [CP4 EPSPS and *gox* genes]. Corn line MON 809 contains one I-DNA of approximately 23 Kb which includes either complete or partial genes of *cryIA(b)*, CP4 EPSPS and *gox*. The I-DNA contains two *cryIA(b)* genes, one which is the correct size, (3.46 Kb), and one which is smaller (less than 1.0 Kb). We conclude that the intact *cryIA(b)* gene produces the detectable CryIA(b) protein and that the partial *cryIA(b)* gene does not produce a protein product at detectable levels. The latter conclusion is based on the size of the partial *cryIA(b)* gene and the lack of production of any detectable protein of the predicted size that cross-reacts with the antibodies to CryIA(b) protein by western blot analysis. There are two CP4 EPSPS genes, both of expected size (1.3 Kb). Western blot analyses demonstrated that the predicted size CP4 EPSPS protein is produced. The single *gox* gene present in corn line MON 809 is not intact. Western blot analysis shows no detectable GOX protein is produced in corn line MON 809, which is consistent with the absence of a full length *gox* gene. Any significant truncated or fusion protein would be expected to be detected

by western blot analysis since: 1) polyclonal antibodies were used which recognize multiple antigenic epitopes on the protein; 2) denatured proteins are detected using western blot analysis, which exposes linear epitopes; and 3) a wide size range of proteins are typically detected, indicating that truncated or fusion protein products could be detected. The *nptII* and *ori-pUC* probings showed that the plasmid backbone was present in the YieldGard corn line MON 809, but was not the predicted size. Based on these analyses, we conclude that corn line MON 809 contains a single I-DNA with an intact *cryIA(b)* gene and two CP4 EPSPS genes that are responsible for producing the correct size CryIA(b) and CP4 EPSPS proteins.

Summary of Corn Line MON 809 Molecular Analysis

Genetic Element	23 Kb insert
cryIA(b) gene	1 full length, 1 partial
CP4 EPSPS gene	2 full length
gox gene	1 partial
nptII / ori-pUC	present

B. Molecular Analysis of YieldGard Corn Line MON 810

This summary describes the molecular analysis of the integrated DNA in YieldGard corn line MON 810. Specifically, the insert number (number of integration sites within the maize genome) and the number and integrity of the inserted genes were determined. Corn line MON 810 was produced by particle acceleration technology using a DNA solution containing two plasmids, PV-ZMBK07 [c*ryIA(b)* gene] and PV-ZMGT10 [CP4 EPSPS and *gox* genes]. The *nptII* gene as cited above was under the control of a bacterial-specific promoter. Molecular analysis of corn line MON 810 established that the line only contains the *cryIA(b)* gene from plasmid PV-ZMBK07. The line does not contain the CP4 EPSPS, *gox*, or *nptII* genes. There is no evidence that any of the DNA contained in plasmid PV-ZMGT10 was inserted. Maize line MON 810 contains one integrated DNA, contained on a 5.5 Kb NdeI fragment, which contains the E35S promoter, the maize hsp70 intron and the *cryIA(b)* gene.

Genetic Element	**Maize Line MON 810**
cryIA(b) gene	present
CP4 EPSPS gene	not present
gox gene	not present
nptII / ori-pUC	not present

C. Segregation Data and Stability of Gene Transfer

1. YieldGard Corn Line MON 809

Segregation data for the R1 plants (derived from selfing the original transformant, or R0 plant), BC0F1 plants (derived from crossing the R0 with an inbred line), BC0F2 plants (derived from selfing the BC0F1 plants), and BC1F1 plants (derived from crossing the BC0F1 plants to the same inbred used to cross with the R0 plant) are presented in Table IV.1. The results in all four cases are consistent with a single active insert segregating according to Mendelian genetics.

Table IV.1 Segregation data and analysis of progeny of YieldGard corn line MON 809

Generation	Actual	Expected	Chi Sq
R1[1]	18:9	13.5:13.5	1.000 *
BC0F1[1]	8:2	5:5	3.600 *
BC0F2[1]	38:12	37.5:12.5	0.000 *
BC1F1[1]	47:50	48.5:48.5	0.041 *

[1] Data expressed as number of expressing plants: number of non-expressing plants based on European corn borer feeding assay

*not significant at p = 0.05 (chi square = 3.84, 1 df)

The *cryIA(b)* gene in YieldGard corn line MON 809 was shown to be stable for five generations of crosses to one recurrent parent (B73) and four generations of crosses to a second, unrelated inbred (Mo17) (Table IV.2). The Chi square tests for the backcross to B73 and to Mo17 did not deviate from expectations at p=0.05.

Table IV.2 Stability of gene transfer based on segregation data for backcross derivatives of YieldGard corn line MON 809 in two unrelated inbred lines (B73 and Mo17). Values are ratios of plants that are positive or negative for the CryIA(b) protein as determined by ELISA.

Generation	Actual	Expected	ChiSq
BC4F1(B73) [1]	20:18	19:19	0.026 *
BC3F1(Mo17) [1]	19:11	15:15	1.633 *

[1] Data expressed as number of expressing plants: number of non-expressing plants based on CryIA(b) ELISA

* not significant at p = 0.05 (chi square = 3.84, 1 df)

To summarize the segregation and stability data (Tables IV.1 and IV.2), the data are consistent with a single active site of insertion of the *cryIA(b)* gene into genomic DNA of line MON 809. The stability of this insertion has been demonstrated through five generations of crossing.

2. YieldGard Corn Line MON 810

Segregation data for the BC0F1 plants (derived from crossing the R0 with an inbred line), BC1F1 plants (derived from crossing the BC0F1 plants to the same inbred used to cross with the R0 plant), and BC1F2 progeny (derived from crossing individual BC0F2 plants by a non-transgenic tester and analyzing subsequent generation ear to row) are presented in Table IV.3. The results are consistent with a single active insert segregating according to Mendelian genetics.

Table IV.3 Segregation data and analysis of progeny of YieldGard corn line MON 810

Generation	Actual	Expected	ChiSq
BC0F1[1]	44:47	45.5:45.5	0.044 *
BC1F1[2]	10:4	7:7	1.786 *
BC1F2 progeny [3]	69:181:77	81.75:163.5:81.75	4.138 #

[1] Data expressed as number of expressing plants: number of non-expressing plants based on European corn borer feeding assay

[2] Data expressed as number of expressing plants: number of non-expressing plants based on CryIA(b) ELISA

[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 European corn borer feeding assay

*not significant at p = 0.05 (chi square = 3.84, 1 df)

not significant at p = 0.05 (chi square = 5.99, 2 df)

The *cryIA(b)* gene in YieldGard corn line MON 810 has been shown to be stable through seven generations of crosses to one recurrent parent (B73) and six generations of crosses to a second, unrelated inbred (Mo17) (Table IV.4). The Chi square tests for the backcross to B73 and to Mo17 did not deviate from expectations at p=0.05.

Table IV.4 Stability of gene transfer based on segregation data for backcross derivatives of YieldGard corn line MON 810 in two unrelated inbred lines (B73 and Mo17). Values are ratios of plants that are positive or negative for the CryIA(b) protein as determined by ELISA.

Generation	Actual	Expected	ChiSq
BC6F1(B73) [1]	8:13	10.5:10.5	0.762 *
BC5F1(Mo17) [1]	11:11	11:11	0.045 *

[1] Data expressed as number of expressing plants: number of non-expressing plants based on CryIA(b) ELISA

* not significant at p = 0.05 (chi square = 3.84, 1 df)

To summarize the segregation and stability data (Tables IV.3 and IV.4), the data are consistent with a single active site of insertion of the *cryIA(b)* gene into genomic DNA of line MON 810. The stability of this insertion has been demonstrated through seven generations of crossing.

D. Conclusion

YieldGard corn lines MON 809 and 810 were produced by particle acceleration technology with a DNA solution containing two plasmids, PV-ZMBK07 (which contained the *cryIA(b)* gene) and PV-ZMGT10 (which contained the CP4 EPSPS and *gox* genes). Corn line MON 809 contains one integrated DNA of approximately 23 Kb which includes a complete (3.46 Kb) and a partial (less than 1.0 Kb) *cryIA(b)* gene, two CP4 EPSPS genes, both of expected size (1.3 Kb), and partial *gox* gene. The *nptII* and *ori-pUC* genes are present but not the predicted size.

Maize line MON 810 contains one integrated DNA contained on an approximately 5.5 Kb NdeI fragment which contains a single copy of the E35S promoter, the *hsp70* intron and the *cryIA(b)* gene. The *nptII* gene and backbone sequences of plasmid PV-ZMBK07 were not integrated. This line does not contain the CP4 EPSPS, *gox*, or *nptII* genes, nor the plasmid backbone from plasmid PV-ZMGT10.

The segregation and stability data for both MON 809 and 810 are consistent with the stable introduction at a single site of insertion of the *cryIA(b)* gene into the genomic DNA of corn.

Part V. Detailed Description of the Phenotype of YieldGard Corn Lines MON 809 and 810

Introduction

Data and information supplied in this Petition for Determination of Non-Regulated Status demonstrate that YieldGard corn lines MON 809 and 810 are substantially equivalent to non-modified corn, except for the inserted genetic sequences, the expressed protein(s), and the ability of the plant to resist damage from certain lepidopteran insects including European corn borer. The information supplied in this section and referenced from USDA Petition 95-093-01p demonstrates that the modified, YieldGard corn lines MON 809 and 810 are 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 inserted 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 lines and to establish that YieldGard corn lines MON 809 and 810 are substantially equivalent to non-modified corn. The inserted genetic material in MON 809 and 810 was described in the previous sections (Parts III and IV). Summaries previously presented in USDA Petition 95-093-01p in support of line MON 80100 include:

- description of the CryIA(b) and CP4 EPSPS proteins including donor organisms
- 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 for genetically modified corn

Other phenotypic descriptions unique to MON 809 and 810 which are described in Part V are as follows:

- expression of CryIA(b), CP4 EPSPS, GOX, and NPTII proteins
- field germination results

- disease and pest susceptibility
- yield characteristics
- the comparison of MON 809 and 810 and parental controls based upon compositional analyses

The following sections summarize these investigations.

A. Expression Levels of the CryIA(b), CP4 EPSPS, GOX, and NPTII Proteins

Levels of the expressed proteins were evaluated in young leaf, grain, whole plant, and pollen tissues collected from six field locations during the 1994 growing season using Enzyme Linked Immuno-Sorbent Assay (ELISA) (Harlow and Lane, 1988) and western blot methods (Matsudaira, 1987). 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 (Sanders *et al.*, 1995). The approximate expression levels are shown in the following table.

Table V.1 Summary of specific protein levels measured in tissues of YieldGard corn lines MON 809 and 810[1]

Corn line	Protein	Leaf	Grain	Whole plant[2,3]	Pollen[2]
		-µg/g fresh weight-			
MON 809	CryIA(b)	1.63	0.55	1.23	N.D.[4]
	CP4 EPSPS	21.68	9.41	1.60	N.A.[5]
	GOX	N.D.	N.D.	N.D.	N.A.
	NPTII	N.A.	N.A.	N.A.	N.A.
MON 810	CryIA(b)	9.35	0.31	4.15	0.09
	CP4 EPSPS	N.D.	N.D.	N.D.	N.A.
	GOX	N.D.	N.D.	N.D.	N.A.
	NPTII	N.A.	N.A.	N.A.	N.A.

1: Values are means calculated from the analyses of six plant samples, one from each of six field sites, unless noted otherwise.

2: The mean was calculated from the analyses of plant sample(s) from one site.
3: Values are means calculated from the analyses of two replicate plant samples from one site.
4: Not detected
5: Not analyzed

B. Field Germination Results

Germination tests of YieldGard maize lines and controls were conducted at six field locations in 1994 across the midwestern U.S. in Illinois, Iowa, Indiana, and Nebraska (Sanders *et al.*, 1995). Results of these tests showed that all seed samples demonstrated high rates of germination, no differences were observed between lines MON 809 and 810 and the control in a variety of environmental conditions (Table V.2). These findings as well as those reported in USDA Petition 95-093-01p for line MON 801 support the conclusion that there are no differences in germination or dormancy between YieldGard corn lines and the non-modified plants.

Table V.2 Field germination results for YieldGard corn lines MON 809 and 810 and control.

Line	Mean germination	Range
MON 809	92.2%	86.7-99.2%
MON 810	87.1%	71.1-94.3%
Control	90.6%	78.9-98.3%

C. Disease and Pest Susceptibilities

YieldGard corn lines MON 809 and 810 have been tested in the United States in over sixty plantings in at least ten states in addition to Puerto Rico since field trials were first established with MON 809 in 1992. Detailed monitoring for the disease and insect susceptibility of these lines versus non-transgenic control plants were performed in 1992 (MON 809 only), 1993 (MON 809 and 810), and 1994 (MON 809and 810) at the sites listed in Tables V.5 and V.6. No differences in agronomic quality, disease, or insect

susceptibility other than European corn borer control were detected between lines MON 809 and 810 and non-transgenic plants. Diseases observed included northern leaf blight (*Exserohilum turcicum*), southern leaf blight (*Bipolaris maydis*), bacterial leaf blight (*Erwinia stewartii*), common corn smut (*Ustilago maydis*), maize stripe virus and common maize rust (*Puccinia sorghi*).

These observations were obtained by comparing the general vigor and disease and insect susceptibility of MON 809 and 810 with non-transgenic lines.

D. Yield Characteristics

Yield comparisons for MON 809, MON 810, and representative controls were assessed in up to nine locations in Iowa, Illinois, Indiana, Nebraska, Ohio and Pennsylvania. The respective gene insertion has been shown not to negatively affect yield. A comparison of a non-transgenic hybrid with the same hybrid in which one parent was a backcross derived MON 809 or 810 line showed no significant difference between these hybrids in yield (Table V.3). YieldGard corn lines MON 809 and 810 that are the subject of this Determination for Non-regulated Status are still in development. Only those lines with commercially acceptable yield and quality characteristics will enter the marketplace.

Table V.3. Yield comparison (bushels/acre) of non-transgenic and MON 809 and 810 versions of the same hybrid.

	MON 809	MON 810
Control	162.3	147.1
YieldGard version	156.5	154.9
difference (pos.- neg)	(5.8)	7.8
standard error	±3.0	±4.5
t-value	-1.987	1.738
p-value	0.052	0.089
difference significant	NO	NO
# of pairs compared	52	44

E. Composition Analysis of YieldGard Corn Lines MON 809 and 810

Compositional parameters of the grain from YieldGard corn lines MON 809 and MON 810 were compared to grain from the parental corn line, MON 818. Grain was harvested from the 1994 GLP field trials at 6 geographically distinct locations in the midwest and analyzed for proximate components: protein, fat, ash, carbohydrate, calories and moisture (Sanders and Patzer, 1995).

Table V.4 summarizes the results of the analyses of grain from corn lines MON 809, MON 810, and MON 818 (control). Protein, fat, ash, and moisture values for both the YieldGard and control lines were similar and within the previously reported ranges (Watson, 1987; Jugenheimer, 1976; Flick, 1995; Lotstein, 1995). Based on these data, it was concluded that the YieldGard corn lines, MON 809 and MON 810, and the control line, MON 818, are substantially equivalent in composition.

Table V.4 **Summary of proximate analysis of grain from corn lines MON 809, MON 810, and 818 (control).**

Characteristic	MON 818[b]	MON 809[b]	MON 810[b]	Reported Range
Protein[a]	12.8	13.1	13.1	6.0-12.0[c] 9.7-16.1[d] 6.8-13.4[e] 10.0-14.1[f]
Fat[a]	2.9	2.6	3.0	3.1-5.7[c] 2.9-6.1[d] 2.0-5.9[e] 1.0-5.7[f]
Ash[a]	1.5	1.5	1.6	1.1-3.9[c]
Carbohydrate[a]	82.7	82.8	82.4	not reported
Calories/100g[a]	409	407	408	not reported
Moisture %	12.0	13.2	12.4	7-23[c]

a: Percent dry weight of sample.
b: Value reported is mean of six samples, one sample from each field site.
c: Watson, 1987.
d: Jugenheimer, 1976.
e: Flick, 1995.
f: Lotstein, 1995

Table V.5 **Disease and insect susceptibility of YieldGard corn line MON 809 in comparison to non-modified corn plants.**

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1992		
Kekaha, HI (92-209-02)	no	no
1993		
Kaunakakai, HI (92-209-03)	no	no
Kihei, HI (92-265-01)	no	no
Jerseyville, IL (93-012-04)	no	no
Monmouth, IL (93-012-04)	no	no
Aurora, IL (93-021-05)	no	no
Bloomington, IL (93-021-06)	no	no
Dekalb, IL (93-021-07)	no	no
Williamsburg, IA (93-021-08)	no	no
Salinas, PR (93-144-02N)	no	no
Kekaha, HI (93-146-02N)	no	no
Kekaha, HI (93-245-02N)	no	no
Kaunakakai, HI (93-258-04N)	no	no
Kaunakakai, HI (93-279-04N)	no	no
Kaunakakai, HI (93-308-02N)	no	no
1994		
Isabela, PR (93-306-04N)	no	no
Kaunakakai, HI (93-316-04N)	no	no
Kunia, HI (93-354-06N)	no	no
Kaunakakai, HI (94-026-04N)	no	no
Santa Isabel, PR (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
Shirley, IL (94-074-12N)	no	no

Table V.5 **Disease and insect susceptibility of YieldGard corn line MON 809 in comparison to non-modified corn plants (continued).**

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1994 - continued		
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 (94-171-05N)	no	no
Santa Isabel, PR (94-171-05N)	no	no
Kaunakakai, HI (94-279-03N)	no	no
Santa Isabel, PR (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 V.5 **Disease and insect susceptibility of YieldGard corn line MON 809 in comparison to non-modified corn plants (continued).**

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1994 - continued		
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

Table V.6 **Disease and insect susceptibility of YieldGard corn line MON 810 in comparison to non-modified corn plants.**

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1993		
Jerseyville, IL (93-012-04)	no	no
Monmouth, IL (93-012-04)	no	no
Kekaha, HI (93-245-02N)	no	no
Loxley, AL (93-250-04N)	no	no
Kaunakakai, HI (93-258-04N)	no	no
Kaunakakai, HI (93-279-04N)	no	no
Kaunakakai, HI (93-308-02N)	no	no
1994		
Isabela, PR (93-306-04N)	no	no
Kaunakakai, HI (93-316-04N)	no	no
Kunia, HI (93-354-06N)	no	no
Kaunakakai, HI (94-026-04N)	no	no
Santa Isabel, PR (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
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

Table V.6 **Disease and insect susceptibility of YieldGard corn line MON 810 in comparison to non-modified corn plants (continued).**

Year/site/ USDA permit/notification no.	Difference in susceptibility versus non-modified corn plants	
	Disease	Insect
1994 - continued		
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
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 (94-171-05N)	no	no
Santa Isabel, PR (94-171-05N)	no	no
Kaunakakai, HI (94-279-03N)	no	no
Santa Isabel, PR (94-279-03N)	no	no
Center Point, IA (94-024-03N)	no	no
Vinton, IA (94-024-03N)	no	no
Johnston, IA (94-024-03N)	no	no
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
Lancaster, PA (94-024-09N)	no	no

F. References

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Part VI. Statement of Grounds Unfavorable

We know of no unfavorable grounds associated with YieldGard corn lines MON 809 and 810. Therefore, on the basis of the substantial potential benefits to the grower, the environment, and the consumer, Monsanto Company requests that this corn line no longer be regulated under 7 CFR part 340.6.

Monsanto

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December 22, 1995

Dr. Vedpal Malik
Biotechnology Permit Unit
Biotechnology, Biologics and Environmental Protection
USDA-APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Dear Dr. Malik:

This letter has been prepared in response to our conversation this previous week regarding Monsanto's USDA petition for non-regulated status for additional corn lines MON 809 and MON 810 received by the USDA on January 17, 1996 and identified as petition 96-017-01p. These lines were previously identified in USDA petition 95-093-01p which provided non-regulated status for line MON 80100 dated August 22, 1995 (FR 60:171; pp. 46107-46108).

Monsanto is requesting that the previous determination for non-regulated status for MON 80100 include corn lines MON 809 and 810 based upon the additional data provided in petition 96-017-01p. This request is based upon the fact that these additional corn lines were produced with a DNA solution containing the same plasmid vectors, PV-ZMBK07 and PV-ZMGT10, as used in the production of MON 80100. In as much, the genes, promoters, and termination sequences utilized in the production of MON 809 and 810 were identical. Differences which exist as to the molecular biology and phenotypic descriptions of MON 809 and 810 when compared to MON 80100 are described in Parts IV and V of recent petition 96-017-01p.

Additionally, Monsanto will provide additional Southern data in support of the molecular analyses provided for corn lines MON 809 and 810 in Part IV of the application as requested by the agency.

Sincerely,

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