Petition for the Determination of Nonregulated Status for MON 88017 Corn

The undersigned submits this petition under 7 CFR Part 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

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Revised

Confidential Business Information-deleted

Release of Information

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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 all relevant data and information known to the petitioner that are unfavorable to the petition.

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Summary

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) and the Plant Quarantine Act (7 U.S.C. § 151-167), to prevent the introduction and dissemination of plant pests into the United States. The APHIS regulation 7 CFR Part 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for MON 88017 corn, any progeny derived from crosses between MON 88017 and other corn, and any progeny derived from crosses of MON 88017 with other genetically-enhanced corn that also has been granted nonregulated status under 7 CFR Part 340.

MON 88017 plants, developed by the use of recombinant DNA techniques, are tolerant to the action of the Roundup® family of agricultural herbicides and are protected from damage caused by corn rootworm (CRW) larval feeding. MON 88017 produces a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium sp.* strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides, and a modified *Bacillus thuringiensis* (subspecies *kumamotoensis*) Cry3Bb1 protein that selectively controls CRW species. The U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA) have previously reviewed the safety of the CP4 EPSPS and Cry3Bb1 proteins present in other biotechnology-enhanced corn products. A synopsis of these safety assessments is provided to the USDA in this petition.

Corn (*Zea mays* L.) is the largest crop grown in the United States in terms of acreage planted and net value. In 2003, corn production covered 79.1 million acres that yielded 10.1 billion bushels and had a net value of $24.8 billion. Weed control is essential in cornfields, as weeds compete with the crop for sunlight, water and nutrients. Failure to control weeds results in decreased yields and reduced crop quality. Introduction of MON 88017 will offer U.S. farmers the ability to apply Roundup agricultural herbicides over the top of corn for broad-spectrum weed control with minimal risk of crop injury. In addition, the use of glyphosate in conjunction with MON 88017 will allow the grower to utilize reduced tillage techniques, which provide significant environmental benefits, such as reduced soil erosion, reduced use of fossil fuels, and improved soil quality.

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Corn yields are also negatively impacted by a number of insect pests. One of the most pernicious in the U.S. corn belt is corn rootworm. Corn rootworm larvae damage corn by feeding on the roots, reducing the ability of the plant to absorb water and nutrients from soil, and causing harvesting difficulties due to plant lodging. Corn rootworm is the most significant insect pest problem for corn production in the U.S. corn belt from the standpoint of chemical insecticide usage, as over 14 million acres of corn were treated with organophosphate, carbamate, and pyrethroid insecticides to control corn rootworm in 2000. Corn rootworm has been described as the billion-dollar pest complex based on costs associated with the application of soil insecticides and crop losses due to pest damage. Introduction of MON 88017 will offer U.S. farmers a safe and effective alternative to the use of synthetic chemical insecticides for control of the corn rootworm pest.

Corn hybrids containing both the corn rootworm protection and glyphosate tolerance traits have been developed previously by crossing two inbreds containing the individual traits by traditional breeding techniques to produce the stacked trait product. However, the traditional breeding process can be inefficient, requiring long development times. MON 88017 was therefore developed using a transformation vector that contained both the *cp4 epsps* and *cry3Bb1* genes. This approach enables the simultaneous introduction of both traits into corn hybrids, thereby providing growers access to a variety of elite corn germplasms containing both traits.

MON 88017 was produced by *Agrobacterium*-mediated transformation of corn cells with plasmid vector PV-ZMIR39. This plasmid contains a gene encoding the CP4 EPSPS protein that provides tolerance to the action of Roundup agricultural herbicides and a gene encoding the Cry3Bb1 protein that has activity against corn rootworm. The *Agrobacterium tumefaciens* transformation vector is a disarmed, binary vector that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation. The DNA region that was integrated into the corn genome during the transformation process contains the *cp4 epsps* and *cry3Bb1* gene expression cassettes. Molecular analyses of MON 88017 confirmed that single copies of the *cp4 epsps* and *cry3Bb1* genes are integrated at a single locus in the corn genome with all expression elements intact and no plasmid bacterial backbone present.

Segregation analysis across ten generations confirmed the heritability and stability of the *cp4 epsps* and *cry3Bb1* coding sequences. The results of this analysis are consistent with the finding of a single active site of insertion that segregates according to the Mendelian laws of genetics.

The CP4 EPSPS protein in MON 88017 is structurally homologous to EPSPSs naturally present in food crops (e.g., soybean and corn) and in microbial food sources such as bakers yeast. The amino acid sequence of the CP4 EPSPS protein produced in MON 88017 is the same or >99% identical to the CP4 EPSPS protein produced in commercial Roundup Ready® crops, such as soybean, NK603 corn, cotton, and canola, which have been granted deregulated status by the USDA.

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A panel of analytical tests was used to characterize and confirm the identity of the CP4 EPSPS protein produced in MON 88017. An evaluation of the CP4 EPSPS protein based on its similarity to natural EPSPS proteins that are ubiquitous in the environment, lack of toxicity in laboratory investigations of Roundup Ready crops against nonpest and pest species, and no adverse effects to human or animal health indicates that the CP4 EPSPS protein produced in MON 88017 will have a no significant impact on the environment.

Bacillus thuringiensis (B.t.) Cry proteins also have a long history of safe and widespread use. The Cry3Bb1 protein produced in MON 88017 is a member of the Cry3Bb class of proteins and shares >99% amino acid sequence identity with the wild type Cry3Bb1 protein contained in the topically applied commercial microbial product, Raven® Oil Flowable Bioinsecticide. The amino acid sequence of the Cry3Bb1 protein produced in MON 88017 is >99.8% identical (a difference of a single amino acid) to that of the Cry3Bb1 protein produced in YieldGard® Rootworm corn (MON 863), which has been deregulated by the USDA, completed the FDA consultation process, and been granted registration by the EPA. The Cry3Bb1 proteins have been exempted from the requirement of a tolerance in corn by the EPA.

A panel of analytical tests was used to characterize and confirm the identity of the Cry3Bb1 protein produced in MON 88017. The environmental assessment of the Cry3Bb1 protein is based on the extensive ecological hazard assessment previously conducted for MON 863. The previous assessment included the testing of several Cry3Bb1 protein variants on representative species of bird, fish and terrestrial nontarget insects, estimation of soil dissipation times, and evaluation of the impact on endangered species and soil microorganisms. Theoretical estimates of margins of exposure (MOEs) for nontarget organisms (NTOs) exposed to the Cry3Bb1 protein produced in MON 88017 show that they are in a similar range to those observed for MON 863. Thus, it is concluded that the Cry3Bb1 protein produced in MON 88017 poses no adverse risk to nontarget organisms and the environment.

Tissue samples of MON 88017 collected from field trials conducted at three sites in the U.S. in 2002 were analyzed by enzyme-linked immunosorbent assay (ELISA) to determine the levels of the CP4 EPSPS and Cry3Bb1 proteins produced in MON 88017. Results showed that the mean CP4 EPSPS protein levels across all sites were 220 µg/g dwt in young leaf, 390 µg/g dwt in pollen, 57 µg/g dwt in forage, 70 µg/g dwt in forage root, and 5.8 µg/g dwt in grain. In tissues harvested throughout the growing season, mean CP4 EPSPS protein levels across all sites ranged from 150-220 µg/g dwt in leaf and 70-150 µg/g dwt in root. In general, levels of the CP4 EPSPS protein declined over the growing season. The levels of the CP4 EPSPS protein produced in tissues of MON 88017 are similar to the levels of the CP4 EPSPS protein produced in Roundup Ready corn NK603.

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The mean Cry3Bb1 levels produced in MON 88017 across all sites were 570 µg/g dwt in young leaf, 25 µg/g dwt in pollen, 380 µg/g dwt in silk, 95 µg/g dwt in forage, 130 µg/g dwt in forage root, 15 µg/g dwt in grain, and 88 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry3Bb1 protein levels across all sites ranged from 260-570 µg/g dwt in leaf, 220-500 µg/g dwt in the whole plant, and 100-370 µg/g dwt in root tissues. In general, levels of the Cry3Bb1 protein declined over the growing season. The levels of the Cry3Bb1 protein produced in tissues of MON 88017 are similar to the levels of the Cry3Bb1 protein produced in YieldGard Rootworm corn (MON 863).

A comprehensive phenotypic and ecological assessment was conducted for MON 88017, which included the evaluation of characteristics for dormancy and germination, emergence, vegetative and reproductive growth, seed retention, and disease, insect, and abiotic stressor-plant interactions. Results from the evaluation of five seed dormancy and germination parameters showed that isolated differences between MON 88017 and control seed were not consistently observed across temperature regimes or production locations, indicating that there is no change in the pest potential of MON 88017 from increased dormancy through hard seed.

The evaluation of 14 phenotypic characteristics, conducted at 18 locations over two years (2001 and 2002), showed no consistent trends in differences between MON 88017 and control corn, other than for seedling vigor. The statistically significant differences observed for seedling vigor across sites in 2001 and 2002 were small in magnitude and not accompanied by consistent across-site differences in stand count, days to pollen shed, or days to silk. This indicates that the differences observed for seedling vigor are unlikely to be biologically meaningful. The evaluation of ecological interactions at the same locations, based on monitoring of specific insect, disease, and abiotic stressors such as heat and drought, showed that there were no detectable trends for differences in susceptibility to pests or to environmental stress. The phenotypic and ecological data indicate that MON 88017 does not confer any detectable selective advantage to corn that would result in increased weed or pest potential compared to control corn.

An environmental assessment of MON 88017 was conducted to assess the impact of the introduced CP4 EPSPS and Cry3Bb1 proteins, the potential for gene flow, and weediness potential of MON 88017. As discussed above, no significant impact on the environment is expected from the CP4 EPSPS and Cry3Bb1 proteins produced in MON 88017.

An assessment of the potential for gene flow indicates that MON 88017 is expected to be similar to conventional corn. Thus pollen-mediated gene flow is expected to occur only with cultivated *Zea* species but not with wild relatives such as *Tripsacum* species that occur in some regions of the U.S. but do not hybridize readily with cultivated corn. An assessment of weed potential based on the results of the comparative studies conducted between MON 88017 and conventional control corn for dormancy, germination, phenotypic and pollen morphology characteristics indicate that MON 88017 is no more likely to become a weed than conventional corn.

An assessment of the impact on current corn agronomic practices indicates that MON 88017 will not impact current cultivation and rotational practices or the managment of insects and diseases other than the control of corn rootworm larvae. The use of MON 88017 will also allow for the broad-spectrum control of grass, broadleaf and perennial weeds by over-the-top applications of Roundup agricultural herbicides, similar to that achieved with Roundup Ready corn NK603. No impact is expected in the management of volunteer corn because there are a number of herbicides labeled for rotational crops that have a mode of action that is different than that of glyphosate.

MON 88017 was shown to be substantially equivalent to conventional corn with a similar genetic background as well as to other conventional corn varieties. Forage and grain tissues were harvested from replicated field trials conducted in the U.S. in 2002. Seventy-seven components were evaluated as part of the nutritional assessment of MON 88017: nine in forage and 68 in grain. Sixty-two components were statistically assessed, as values for 15 components were below the limit of quantitation. Results of the analysis showed that there were no statistically significant differences between MON 88017 and conventional corn for 232 of the 248 comparisons conducted. The test values for each statistically significant comparison ($p<0.05$) fell within the 99% tolerance interval, historical control ranges, and literature ranges. Therefore it is unlikely that these minor differences are biologically meaningful. It is concluded, based on these data, that the forage and grain produced from MON 88017 are compositionally equivalent to the forage and grain produced from other commercial corn currently on the market.

Data and information presented in this request demonstrate that MON 88017 does not pose a unique plant pest risk. Therefore Monsanto Company requests that APHIS grant the request for a determination of nonregulated status for MON 88017.

Abbreviations, Acronyms and Definitions

~	Approximately
AA	Amino acid
aad	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase, from the transposon Tn7
ADF	Acid detergent fiber
ALS	Acetolactate synthase
ANOVA	Analysis of Variance
AOSA	Association of Official Seed Analysts
Avg	Average
B	Border region
BSA	Bovine serum albumin
B.t.	*Bacillus thuringiensis*
CaMV	Cauliflower Mosaic virus
CFR	Code of Federal Regulations
CI	Confidence interval
CPB	Colorado potato beetle
CRW	Corn Rootworm (*Diabrotica* species)
Cry	Crystal proteins from *B.t.*
Cry3	A class of *B.t.* crystal proteins with insecticidal activity against coleopteran species
Cry3Aa4	A *B.t.* crystal protein with activity against the Colorado potato beetle
cry3Bb1	Nucleotide coding sequence for the Cry3Bb1 protein
Cry3Bb1	A naturally-occurring wild type protein with activity against coleopteran insects, produced by *B.t.k*
CP4	*Agrobacterium sp.* strain CP4
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* species strain CP4
cp4 epsps	Coding sequence for the CP4 EPSPS protein from *Agrobacterium sp.* strain CP4 present in PV-GHGT35
CTP	Chloroplast transit peptide
CTP2	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS
CTAB	Cetyltrimethylammonium bromide
CV	Coefficient of variation
DAP	Days after planting
dATP	Deoxyadenosine triphosphate

Abbreviations, Acronyms and Definitions (cont'd.)

dCTP	Deoxycytidine triphosphate
df	Degrees of freedom
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
DWCF	Dry weight conversion factor
dwt	Dry weight
e35S	Enhanced 35S plant promoter
ECL	Enhanced chemiluminescence
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
FA	Fatty acid
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FFDCA	Federal Food, Drug and Cosmetic Act
FW	Fresh weight
Fwt	Fresh weight of tissue
GDU	Growing degree units
GLP	Good Laboratory Practice
HCl	Hydrochloric acid
HEPES	N-[2-(Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)]
HPLC	High performance liquid chromatography
HRAC	Herbicide Resistance Action Committe
HRP	Horseradish peroxidase
HSD	Honestly Significantly Difference
I	Intron
IgG	Immunoglobulin G
IPM	Integrated Pest Management
IUPAC-IUB	International Union of Pure and Applied Chemistry - International Union of Biochemistry
KCl	Potassium chloride
LB	Left border
LOQ	Limit of quantitation
LOD	Limit of detection
MALDI-TOF	Matix-assisted laser desorption ionization time of flight
$MgCl_2$	Magnesium chloride
MH+	Protonated mass ion
MEEC	Maximum Expected Environmental Concentration
MMT	Million metric tonnes
MOE	Margin of Exposure
MON 810	A Monsanto corn product, producing an insecticidal *B.t.* Cry1Ab protein

Abbreviations, Acronyms and Definitions (cont'd.)

MON 863	A Monsanto corn product, producing the insecticidal *B.t.* Cry3Bb1 protein and the marker protein *nptII*
MON 88017	A Monsanto corn product, and the subject of this application, producing the glyphosate-tolerant CP4 EPSPS protein and the insecticidal *B.t.* Cry3Bb1 protein
MOA	Mode of action
MS	Mass spectrometry
MW	Molecular weight
MWM	Molecular weight marker
NASS	National Agricultural Statistics Service, a branch of USDA
NCRW	Northern corn rootworm (*Diabrotica barberi*)
NDF	Neutral Detergent Fiber
NK603	A Monsanto corn product producing the glyphosate-tolerant CP4 EPSPS protein
NOEC	No Observable Effect Concentration
NOEL	No Observable Effect Level
NOS	Nopaline synthase 3' polyadenylation sequence
NTO	Nontarget organism
na	Not available
NaCl	Sodium chloride
NaOAc	Sodium acetate
NDF	Neutral detergent fiber
NFDM	Non-fat dried milk
NIST	National Institute of Standards and Technology
OD	Optical density
OSL	Overseason leaf
OSR	Overseason root
OSWP	Overseason whole plant
ori-322	Origin of replication from pBR322 for maintenance of plasmid in *E. coli*
ori-V	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline containing 0.05% (v/v) Tween-20
PCR	Polymerase chain reaction
PEP	Phosphoenolpyruvate
PIP	Plant-incorporated protectant
ppm	Parts per million (µg of analyte/g of sample)
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride

Abbreviations, Acronyms and Definitions (cont'd.)

RB	Right border
PV-ZMIR39	Plasmid vector used for the transformation of corn to produce MON 88017
RDR	Root Damage Rating
ROP	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SE	Standard error
S3P	Shikimate-3-phosphate
Taq	*Thermus aquaticus*, a thermophilic bacterium
tahsp17 3'	3' nontranslated region of the wheat 17.3 kDa heat shock protein containing the polyadenylation sequence
TBA	Tris-borate buffer with L-ascorbic acid
TDF	Total dietary fiber
TE	Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
TFA	Trifluoroacetic acid
TI	Tolerance interval
TMB	3,3',5,5'-tetramethylbenzidene
Tris	Tris(hydroxymethyl)aminomethane
TSSP	Tissue-specific site pool
USDA-APHIS	United States Department of Agriculture – Animal and Plant Health Inspection Service
WCRW	Western corn rootworm (*Diabrotica virgifera virgifera*)
wt CAB	A 5' untranslated leader of the wheat chlorophyll a/b/-binding protein

Standard abbreviations, e.g., units of measure, are used according to the format described in 'Instructions to Authors' in the *Journal of Biological Chemistry*.

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I. Rationale for the Development of MON 88017

I.A. Basis for the Request for a Determination of Nonregulated Status under 7 CFR Part 340.6

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) and the Plant Quarantine Act (7 U.S.C. § 151-167), to prevent the introduction and dissemination of plant pests into the United States. The APHIS regulation 7 CFR Part 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

I.B. Rationale for the Development of MON 88017 and Benefits

This section provides background and rationale for the development of MON 88017, including benefits of the product. The commercialization of MON 88017 will provide growers with the benefits of weed and insect control in one bag of seed. The following subsections describe benefits of weed control based on the experience with Roundup Ready corn and other crops, and the control of corn rootworm larvae by the use of YieldGard Rootworm corn (MON 863), and the benefits specific to MON 88017.

I.B.1. Benefits of Weed Control

Corn is the largest crop grown in the U.S. in terms of acreage planted and net value. In 2003, its production covered 79.1 million acres that yielded 10.1 billion bushels and had a net value of $24.8 billion (USDA-NASS, 1995-2004). Corn yields are negatively impacted by a number of weeds and insect pests.

Corn yield loss is generally proportional to the amount of weeds present. While the ratio is not always one to one, some studies suggest that for every pound of weed dry matter, there is a reduction of approximately one pound of corn dry matter (grain, cobs, stalks and leaves). Competition for light, nutrients, and moisture resources by the crop and weeds can lead to proportional reductions in yield (Knake et al., 1990). Numerous studies have shown that weed control early in the growing season is necessary to reduce yield losses in corn. Weed species such as giant foxtail, barnyardgrass and pigweed can reduce corn yields by up to 13, 35 and 50%, respectively (Bosnic and Swanton, 1997; Fausay et al., 1997; Knake and Slife, 1965). In a study of mixed weed populations competing with corn, corn yields were reduced by up to 20% when the weed plants reached a height of eight inches (Carey and Kells, 1995).

Weed control systems used in U.S. corn production are reviewed in detail in Section VIII. The choice of weed-control methods depends on the particular cropping system (e.g.,

conventional tillage, conservation tillage, no till), local weed spectrum, costs, and other factors. In 1998, Monsanto commercialized Roundup Ready corn GA21, containing the modified maize 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) gene, which offered a biotechnology-derived solution for the control of weeds by the use of over-the-top applications of Roundup agricultural herbicides. This was followed by the commercialization in 2000 of the replacement product Roundup Ready corn NK630 containing the *cp4 epsps* gene, which allowed for a wider application window and slightly higher rates of Roundup agricultural herbicides for enhanced weed control with minimal crop injury.

Use of a Roundup agricultural herbicide in crop provides an efficient and cost effective means of controlling weeds. Roundup agricultural herbicides are used as foliar-applied, nonselective herbicides, and are effective against the majority of annual and perennial grasses and broadleaf weeds. Glyphosate, the active ingredient in Roundup agricultural herbicides, binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of 5-enolpyruvylshikimate-3-phosphate (EPSP), thereby starving plants of essential amino acids and secondary metabolites (Steinrucken and Amrhein, 1980; Haslam, 1993). EPSPS proteins catalyze the transfer of the enolpyruvyl group from phosphoenol pyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding EPSP and inorganic phosphate (Alibhai and Stallings, 2001). In Roundup Ready plants, which are tolerant to Roundup agricultural herbicides, aromatic amino acids and other metabolites that are necessary for plant growth and development are met by the continued action of the inserted glyphosate-tolerant CP4 EPSPS enzyme (Padgette et al., 1996).

Glyphosate has favorable environmental and safety characteristics. Glyphosate has no preemergence or residual soil activity (Franz et al., 1997), is not prone to leaching, degrades in soil over time, and poses minimal risk to mammals, birds or fish under normal conditions of use (EPA, 1993; WHO, 1994; Giesy et al., 2000; Williams et al., 2000). Furthermore, glyphosate has been extensively evaluated in scientific studies that have concluded that glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects or reproductive problems (Williams et al., 2000).

Since its introduction into the marketplace, the Roundup Ready corn system has provided many benefits to growers including:

- *Broad-spectrum weed control*. Roundup agricultural herbicides control both broadleaf weeds and grasses, including difficult to control weed species (Franz et al., 1997).
- *Excellent crop safety*. When used according to label directions, Roundup agricultural herbicides control weeds without injury to Roundup Ready corn plants.
- *Flexibility in treating for weed control*. Because Roundup agricultural herbicides are applied to the foliage of weeds after crop emergence, applications are only necessary if weed infestation reaches the threshold level for yield reductions.
- *Convenience and simplicity*: The Roundup Ready corn system increases farming convenience and production simplicity.

- *High compatibility with Integrated Pest Management (IPM) and soil conservation techniques*. Benefits of conservation tillage include improved soil quality, improved water infiltration, reduced soil erosion and sedimentation of water resources, reduced runoff of nutrients and pesticides to surface water, improved wildlife habitat, increased carbon retention in soil, reduced fuel usage, and use of sustainable agricultural practices (Warburton and Klimstra, 1984; Edwards et al., 1988; Hebblethwaite, 1995; Reicosky, 1995; Reicosky and Lindstrom, 1995; Keeling et al., 1998; CTIC, 1998; CTIC, 2000). In addition, data collected during 1999-2001 have shown that the adoption of Roundup Ready corn can lead to a significant reduction in the levels of major preemergence corn herbicides in Illinois watersheds (Wauchope et al., 2001).
- *Cost effective weed control*. The costs of weed control with Roundup agricultural herbicides are competitive with the cost of alternative weed-control options. Both large- and small-scale farmers benefit equally from use of this technology.
- *Economic benefits*. In assessing the adoption of herbicide-tolerant cultivars by growers in the U.S., Gianessi et al. (2002) noted that growers have substituted traditional herbicides with preemergence and postemergence treatments of Roundup agricultural and other herbicides for the control of problem weeds in corn. It is estimated that these substitutions result in an average reduction in the amount of herbicide used by 1 lb/A, and reduction in costs to the grower of $10/A.
- *Minimal environmental impact.* Roundup agricultural herbicides have been used for more than 30 years in various applications. Glyphosate is recognized for its lack of soil persistence, low risk of ground water contamination, and lack of toxicity to birds, mammals, fish, and invertebrates (Ahrens, 1994; Franz et al., 1997; Giesy et al., 2000).

Although few systems offer optimal performance in all of these areas, use of Roundup Ready corn provides the grower with cost effective, broad-spectrum weed control with safety and environmental benefits resulting from the use of Roundup agricultural herbicides.

I.B.2. Benefits of Corn Rootworm Control

Of the several insect species that can cause damage to corn plants, one of the most pernicious in the U.S. corn belt is larvae of corn rootworm species, northern corn rootworm (NCRW*; Diabrotica barberi*) and western corn rootworm (WCRW*; Diabrotica virgifera virgifera*). These insects damage corn by feeding on the roots, reducing the ability of the plant to absorb water and nutrients from the soil, and causing harvesting difficulties because of plant lodging (Reidell, 1990; Spike and Tollefson, 1991). Corn rootworm is the most significant insect pest problem for corn production in the U.S. from the standpoint of chemical insecticide usage. Approximately 14 million acres of corn were treated in 2000 with organophosphate, carbamate and pyrethroid insecticides to control corn rootworm (Doane, 2001). Corn rootworm has been described as the billion-dollar pest complex, based on costs associated with the application of soil insecticides and crop losses because of pest damage (Metcalf, 1986).

Corn growers mitigate corn rootworm damage primarily through crop rotation or by the use of soil insecticides. However, the usefulness of crop rotation is now limited because of the existence of a NCRW variant that exhibits an extended diapause period, and a WCRW variant that, unlike previous populations, lays its eggs in soybean fields (Gray et al., 1998; Onstad and Joselyn, 1999; O'Neal et al., 1999). The eggs of this WCRW variant over-winter in soybean fields and emerge the following year in corn. Since its initial discovery in Illinois, this variant is expected to rapidly spread throughout the corn belt.

In 2003, Monsanto commercialized MON 863, which provides an alternative and effective solution for the control of corn rootworm larvae based on biotechnology. In granting the registration of MON 863, EPA assessed the potential benefits of corn rootworm protection technology by: a) comparing the efficacy of MON 863 with chemical control options for corn rootworm, b) evaluating the human health and environmental benefits compared to registered insecticides, c) estimating economic benefits to the grower, and d) estimating the chemical pesticide use reduction afforded by the adoption of MON 863. EPA made a determination that the registration of MON 863 was in the public interest and the benefits outweighed the risks (EPA, 2003).

The Cry3Bb1 protein has no toxic effects on NTOs based on results in all appropriate tests and is degraded rapidly in the soil thereby minimizing exposure to NTOs. In addition, the Cry3Bb1 protein has a narrow target range confined to beetles of the family Chrysomelidae that are not known to contain endangered species. To date, no functional receptors for Cry proteins on the intestinal cells of fish, birds, or mammals have been identified. Thus, the use of MON 88017 is likely to pose less risk to the environment than currently registered insecticides for the control of corn rootworms.

Corn rootworm technology offers a significant yield and econonomic advantage for growers (EPA, 2003). Preliminary estimates for MON 863 place this yield benefit at 1.5 to 4.5%. For a reasonable range of prices and yields, the benefit to growers is US$4-12/A relative to the use of a soil-applied insecticide, depending on corn rootworm pressure. The EPA has estimated cumulative grower benefits at $6.56/acre based on expected yield improvements, reduced costs for insecticides, and practical benefits related to a more flexible and safer product for growers to use than the alternatives.

I.B.3. Combined Benefits of Weed and Corn Rootworm Control

The commercialization of MON 88017 will provide growers with the combined benefits of the Roundup Ready corn system and corn rootworm technology. Thus growers will have the ability to apply Roundup agricultural herbicides over the top of corn for broad-spectrum weed control with a minimal risk of crop injury, and control corn rootworm larvae, a major insect pest of corn in the U.S.

Corn hybrids containing both the corn rootworm protection and glyphosate tolerance traits have been developed previously by crossing two inbreds containing the individual traits by traditional breeding techniques to produce the stacked trait product. However, the traditional breeding process can be inefficient, requiring long development times.

MON 88017 was therefore developed using a vector that contains both the *cp4 epsps* and *cry3Bb1* genes. This approach increases the efficiency of simultaneous introduction of both traits into corn, thereby providing growers access to a variety of elite corn germplasms containing both traits. The potential benefits of MON 88017 for weed control will be similar to those of Roundup Ready corn NK603 because it produces the same glyphoste-tolerant CP4 EPSPS protein, and will be similar to MON 863 for corn rootworm control because it produces a Cry3Bb1 protein that is equally efficacious against this target pest.

This leads to the conclusion that commercialization of MON 88017 will provide substantial benefits to growers by limiting yield losses from weed pressure and corn rootworm feeding damage while at the same time reducing the risk to humans and the environment.

I.C. Adoption of MON 88017

Currently, corn growers in the U.S. have a wide range of weed-control options when they make planting decisions, both biotechnology- and conventional-based. Since its introduction in 1998, growers have planted Roundup Ready corn on millions of acres. Similarly, following its introduction in 2003, the adoption of YieldGard Rootworm corn (MON 863) for the control of corn rootworm larvae is expected to increase in the coming years. Growers are expected to adopt MON 88017 in those regions of the U.S where the combination of weed and corn rootworm pressure is significant. The major area of adoption is likely to be the U.S. corn belt including Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota and Wisconsin.

I.D. Submissions to Other Regulatory Agencies

I.D.1. Submission to the FDA

MON 88017 falls within the scope of the Food and Drug Administration's (FDA) policy statement concerning regulation of products derived from new plant varieties, including those produced through genetic engineering (FDA, 1992). Monsanto has voluntarily initiated and will complete a consultation process with FDA prior to commercial distribution of this product. A safety and nutritional assessment of food and feed derived from MON 88017 was submitted to the FDA on March 31, 2004.

I.D.2. Submissions to the EPA

Substances that are pesticides, as defined under the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. §136(u)), are subject to regulation by the Environmental Protection Agency (EPA). Pesticides produced *in planta*, referred to as plant-incorporated protectants (PIPs), are also subject to regulation by the EPA (EPA, 2001).

In January 2004, Monsanto submitted an application to the EPA to register the PIP *Bacillus thuringiensis* Cry3Bb1 protein, and the genetic material (vector PV-ZMIR39)

necessary for its production in MON 88017. An application for an exemption from the requirement of a tolerance for *B.t.* Cry3Bb1 proteins, pursuant to §408(d) of the Federal Food Drug and Cosmetic Act (21 U.S.C. 346 a(d)), was also submitted to EPA (PP 7F4888). On May 11, 2001, EPA established a time-limited exemption from the requirement of a tolerance for Cry3Bb1 and the genetic material necessary for its production in all corn commodities (EPA, 2001). On April 29, 2003, Monsanto petitioned the EPA to amend 40 CFR Part 180 by removing the time limitation for the exemption from the requirement of a tolerance for the PIP *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production in corn in or on field corn, sweet corn, and popcorn. On March 31, 2004, EPA removed the time-limitation for the exemption of a requirement of a tolerance for the Cry3Bb1 protein (EPA, 2004).

Herbicides are also subject to EPA's regulatory authority. The initial registration for use of Roundup Ultra® Herbicide (EPA Reg. No. 524-475) over the top of Roundup Ready corn was granted by the EPA on March 28, 1997 (62 FR 17723-17730). Since that time a number of label amendments have been approved by the Agency, and the use of glyphosate in Roundup Ready corn has been approved for multiple glyphosate end-use product registrations. The most recent label for use in Roundup Ready corn, approved on June 23, 2003, for Roundup WeatherMAX® herbicide (EPA Reg. No 524-537), provides greater flexibility for in-crop applications. This new label allows increased over-the-top application rates and a wider window for applications with the use of directed sprays up to a corn plant height of 48 inches. EPA's approval of the label was based on the review of supporting glyphosate residue data and the establishment of an increased tolerance level of 6 ppm for glyphosate residues in field corn forage (68 FR 39460, July 2, 2003).

Pursuant to section 408(d) of the Federal Food Drug and Cosmetic Act (FFDCA), 21 U.S.C. 346 a(d), the EPA previously has reviewed and established an exemption from the requirement of a tolerance for CP4 EPSPS and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR §180.1174).

I.D.3. Submissions to Foreign Governments

Regulatory submissions for import and production approvals will be made to countries that import U.S. corn grain and have regulatory approval processes in place. These will include submissions to a number of foreign government regulatory agencies, including Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF), the Japanese Ministry of Health, Labor and Welfare (MHLW), as well as the Canadian Food Inspection Agency (CFIA) and Health Canada. As appropriate, notifications of import will be made to importing countries that do not have a formal approval process.

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II. The Corn Family

This section summarizes the biology of corn based on the consensus document for maize prepared by the Organization for Economic Co-operation and Development (OECD, 2003), a summary prepared by USDA-APHIS and available at their website (USDA-APHIS, 2004), information provided in the USDA petition for YieldGard Rootworm corn MON 863 (Petition 01-137-01p), and recent literature publications.

II.A. Corn as a Crop

Corn (*Zea mays* L.), or maize, is one of the few major crop species indigenous to the Western Hemisphere. It is a member of the *Maydeae* tribe of the grass family, *Poaceae*. It is a robust monoecious annual plant, which requires the help of man to disperse its seeds for propagation and survival. Corn is an efficient plant for capturing the energy of the sun and converting it into food, and adapts readily to different conditions of humidity, sunlight, altitude, and temperature.

Corn is grown in nearly all areas of the world and ranks third behind rice (*Oryza sativa* L.) and wheat (*Triticum* sp.) in total global production. In 2002, corn was planted globally on 140 M ha with a total production of 602 million metric tones (MMT) (ISAAA, 2003). The top five production countries in 2002 were: USA (228.7 MMT), China (124 MMT), Brazil (35.5 MMT), Mexico (19 MMT) and France (16 MMT). In the U.S., corn is grown in almost all the states (see Section VIII for additional details), and in 2003, its production value of $24.8B was the highest of any crop.

In industrialized countries corn has two major uses: 1) as animal feed in the form of grain, forage or silage; and 2) as a raw material for wet- or dry-milled processed products such as high fructose corn syrup, oil, starch, glucose, and dextrose (Tsaftaris, 1995). These processed products are used as ingredients in many industrial applications and in human food products. In developing countries, corn is used in a variety of ways. In Latin American countries such as Mexico, one of the main uses of corn is for food. In Africa, corn is consumed as a food in the sub-Saharan region, and in Asia it is generally used to feed animals (Morris, 1998).

Corn has been studied extensively, and it seems probable that the domestication of corn occurred in southern Mexico 7,000 to 10,000 years ago. The putative parents of corn have not been recovered, but it is likely that teosinte 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 know 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 or one difficult to control. A summary of the history, taxonomy, genetics, and life cycle of corn is presented below, followed by a discussion of gene flow between cultivated corn and its wild relatives.

II.B. 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 in November 1492, and introduced corn to Europe upon his return to Spain (Goodman, 1988). Within two generations after the introduction of corn to Europe, corn became distributed throughout those regions of the world where it could be cultivated.

The original corn-growing areas did not include the Northcentral region (U.S. corn belt) of the United States. The highly productive U.S. corn belt dent corn was 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 corn 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 Northeastern North America.

The corn types 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 corn grown in the southeast United States seemed to have originated from the southeast coast of Mexico. Southern dent corn is 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 corn that is used extensively throughout the world today.

The origin of corn has been studied extensively and four main hypotheses have been suggested (OECD, 2003):

1. *Descent from teosinte* - corn originated by direct selection from teosinte;
2. *The tripartite hypothesis*: 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);
3. *The common origin hypothesis* - corn, teosinte, and *Tripsacum* descended independently from a common, unknown ancestor; and,
4. *The catastrophic sexual transmutation hypothesis* – that modern corn originated from teosinte by an epigenetic sexual mutation causing ear development.

Other suggestions have included *Coix* and species of the genus *Manisuris* in the tribe *Andropogoneae* as 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 the use of molecular genetic markers.

Evidence has been reported to support the different hypotheses, but it seems that 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 one to three 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).

II.C. Taxonomy

Corn (*Zea Mays* L.) is a member of the tribe Maydae, which is included in the subfamily Panicoideae of the grass family Gramineae. Table II-1 summarizes the taxonomic classification of corn and its close relatives.

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 some researchers have implicated the Asian genera in the origin of corn, the evidence for them is not as extensive and convincing as for the genera located in the Western Hemisphere.

Table II-1. Taxonomic Classification of Corn and its Close Relatives

Family - Gramineae
- Subfamily - Panicoideae
  - Tribe - Maydae
    - Western Hemisphere:
    - I. Genus - *Zea*
      - A. Subgenus - *Luxuriantes*
        1. *Zea luxurians* (2n = 20)
        2. *Zea perennis* (2n = 40)
        3. *Zea diploperennis* (2n = 20)
      - B. Subgenus - *Zea*
        1. *Zea mays* (2n = 20)
           Subspecies
           1. *Z. mays parviglumis* (2n = 20)
           2. *Z. mays huehuetenangensis* (2n = 20)
           3. *Z. mays mexicana* (Schrad.) (2n = 20)

    - II. Genus – *Tripsacum*
      - A. Section – *Tripsacum*
        Species
        1. *T. andersomii* (2n = 64)
        2. *T. australe* (2n = 36)
           Varieties
           a) *T. australe* var. *australe*
           b) *T. australe* var. *hirstum*
        3. *T. bravum* (2n = 36, 72)
        4. *T. cundinamarce* (2n = 36)
        5. *T. dactyloides* (2n = 72)
           Varieties
           a) *T. dactyloides* var. *hispidum*
           b) *T. dactyloides* var. *dactyloides*
           c) *T. dactyloides* var. *meridonale*
           d) *T. dactyloides* var. *mexicanum*
        6. *T. floridanum* (2n = 36)
        7. *T. intermedium* (2n = 72
        8. *T. manisuroides* (2n = 72)
        9. *T. latifolium* (2n = 36)
        10. *T. percuvianum* (2n = 72, 90, 108)
        11. *T. zopilotense* (2n = 36, 72)
      - B. Section – *Fasciculata*
        Species
        1. *T. jalapense* (2n = 72)
        2. *T. lanceolatum* (2n = 72)
        3. *T. fasciculatum* (2n = 36)
        4. *T. maizar* (2n = 36, 72)
        5. *T. pilosum* (2n = 72)
           Varieties
           a). *T. pilosum* var. *guatemalense*
           b). *T. pilosum* var. *pilosum*

    - Asia:
    - I Genera—
      Chionachne (2n = 20) *Schlerachne* (2n = 20)
      Coix (2n = 10, 20) *Trilobachne* (2n = 20)
      Polytoca (2n = 20)
  - Tribe—Andropogoneae
    - I. Genus - *Manisuris*

The genus *Zea* includes two sections: *Luxuriantes* and *Zea*. Corn (*Zea mays* L.) is a separate species within the subgenus *Zea*, along with three subspecies. All 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 (Table II-1). An additional species, *Tripsacum hermaphrodita* (*Anthephora hermaphrodita*), has been described in the literature but has not been formally included in this taxonomic classification (USDA-NCRS, 2004). In addition to their occurrence in other regions of the Americas, five *Tripasacum* species are native to the U.S. (Texas A&M, 2002). *Tripsacum dactyloides,* or Eastern gamagrass, is the most widely distributed species and can be found in the Midwestern, Eastern and Southern regions of the U.S. The other species have a more limited geographical distribution and can be found in the following states and territories: *Tripsacum floridanum* in Florida, *Tripsacum lanceolatum* in Arizona and New Mexico, *Tripsacum fasciculatum* in Puerto Rico, and *Tripsacum hermaphrodita* in Puerto Rico and the Virgin Islands. Twelve of 16 *Tripsacum* species 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).

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.

II.D. Genetics

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 the 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 those 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, and in gene transformation (Coe et al., 1988; Carlson, 1988; Phillips et al., 1988; Walbot and Messing, 1988).

The corn genome is approximately 5 x 10^6 kb in size (OECD, 2003). It includes highly repeated sequences that constitute about 20% of the genome; these sequences are present in about ten superabundant sequence types. There are more than 1000 different moderately repetitive sequence families, collectively representing 40% of the genome. The remaining 40% of the genome contains single-copy sequences, or more than approximately 10^6 gene-size regions.

II.E. Life Cycle

Corn is an annual plant and the duration of its 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 for more than six to eight hours after the growing point is above ground (five- to seven-leaf stage). Damage from freezing temperatures, however, depends on the extent of temperatures below 0°C, soil condition, amount of residue, the duration 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 that corn with differences in the length of their life cycles can be grown in north-to-south directions of temperate areas. In the United States, corn with relative maturities of 80 days or less are grown in the extreme northern areas, and corn with relative maturities of more than 125 days are grown in the southern areas. Corn having relative maturities of 100 to 115 days is 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 maturity of corn is the number of growing degree units (GDU) required from emergence to maturity. Based on the GDU required to mature, corn is assigned to areas that have, on the average, less than 1850 GDU in the extreme northern areas of the United States to corn that requires more than 2750 GDU in more southern areas. Assume a 115-day maturity hybrid is grown in central Iowa. The average last frost date is May 1 and the 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 of 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 estimated time frames could vary within the same year among locations and among years at the same location, depending on the environmental conditions experienced from planting to harvesting.

II.F. 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, only open-pollinated corn varieties were grown. 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 self pollinating (or inbreeding, which is the opposite of outcrossing) within a corn variety (Shull, 1908). Because corn naturally cross- fertilizes, the genetic composition of each plant is not known. Continuous selfing of individuals for seven to ten 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. Recent techniques such as marker-assisted breeding have reduced the time and number of generations required to produce pure inbreds (Youseff and Juvik, 2001).

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: self to develop pure lines, cross the pure lines to

produce hybrids, evaluate hybrids to determine the best hybrid, and use pure-line parents to reproduce the superior hybrid and distribute it for use by the growers (Shull, 1909).

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, to produce the crosses of superior lines for distribution to growers. Hybridization is easily accomplished either by hand-pollination or by wind-pollination in large crossing fields (male and female inbred lines) to produce large quantities of high-quality hybrid seed.

II.G. Pollination

II.G.1. Outcrossing with Wild *Zea* Species

Annual teosinte (*Zea mays* subsp. *mexicana*) and corn are wind-pollinated, self-compatible, and are highly variable, interfertile species (Wilkes, 1972 and 1989). Corn and teosinte are genetically compatible, and in areas of Mexico and Guatemala they freely hybridize when they are in proximity to each other and other conditions are favorable. Teosinte exists primarily as a weed around the margins of corn fields, and the frequency of hybrids between teosinte and corn has been studied. A frequency of one F_1 hybrid (corn x teosinte) for every 500-corn plants has been reported for the Chalco region of the Valley of Mexico (Wilkes, 1972). The F_1 hybrid of teosinte by corn is robust and fertile and is capable of backcrossing to corn. Intercrossing and gene exchange between teosinte and corn occurs freely, and, accompanied by selection, teosinte had a significant role in the evolution of corn. Recently, Evans and Kermicle (2001) have shown that although corn can introgress into teosinte, there is incompatibility between some corn populations and certain types of teosinte, resulting in low fitness of some hybrids that prevents a high rate of introgression.

Although corn easily crosses with teosinte, 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; Gonzalez and Corral, 1997). Dependent upon the human characterization of teosinte with its local environment, it may be considered a weed. However, it has been noted that populations of teosinte have been in decline for several decades because of increased grazing and urbanization in Mexico (Wilkes, 1995). Except for special plantings, there are no reports of teosinte occurring in the United States.

II.G.2. Outcrossing with *Tripsacum* Species

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, which can be easily hybridized, both in the wild and by controlled pollinations, special techniques are required to hybridize corn and *Tripsacum*. With the exception of *Tripsacum floridanum*, it is difficult to cross *Tripsacum* with corn, and the offspring of the cross show varying levels of sterility. *Tripsacum*-corn hybrids have not been observed in the field, and *Tripsacum*-teosinte hybrids have not been produced (Wilkes, 1972).

In recent years additional research has been conducted on the hybridization of corn with *Tripsacum* species. Eubanks (1995, 1998) has developed a method for transferring *Tripsacum* genes into corn. In this method two wild relatives of corn, *Tripsacum* and diploid perennial teosinte (*Zea diploperennis*), were crossed to produce a hybrid, which is called tripsacorn and can be used to generate corn-tripsacorn hybrids. The use of tripsacorn is intended to confer resistance to pests and disease, drought tolerance, and improved uniformity. Eubanks (2000) has claimed that traits such as apomixis, totipotency, perennialism, adaptation to adverse soil conditions and to a carbon dioxide enriched atmosphere can be transmitted to corn by such techniques. While these discoveries show promise, additional research needs to be conducted to determine their widespread applicability.

In experiments with *Tripsacum dactyloides* or Eastern gamagrass, DeWald et al. at the USDA succeeded in obtaining a true *Tripsacum* cytoplasm with a corn nuclear background (DeWald et al., 1999). In this experiment Eastern gamagrass was used as the female parent and corn as the male or pollen donor. The *Tripsacum*-derived mitochondrial chondrome and chloroplast plastome in these hybrids contribute to the seed qualities of the plants, but the nuclear genome appeared to be totally corn in origin. These results suggest that any possibility of corn contributing genetic material to Eastern gamagrass through random pollen flow in agricultural situations is extremely remote, and if this were to occur, the resultant genome would be lacking in most or all of the corn chromosomal complement.

II.G.3. 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 corn will interpollinate, except for certain popcorn varieties and hybrids that have one of the dent-sterile 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, 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 completes fertilization within 24 hours. Although there may be some minor differences in the rate of pollen germination and pollen tube elongation on some genotypes, corn pollen is 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 seed will be located so that the seed parent is no less than 200 m (~660 feet) 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 corn). 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.

II.H. Weediness of Corn

Modern-day corn cannot survive outside of cultivation (Gould, 1968). One does not find volunteer corn growing in fence rows, ditches, and roadsides as a weed. Although corn from the previous crop year can overwinter and germinate the following year, it 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 often are taken to eliminate either the plants with a hoe or to use herbicides to kill the corn plants in soybean fields, but the plants that remain and produce seed usually do not persist in the following years.

It is difficult for 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 does not occur naturally because of the structure of the ears of corn. Individual kernels of corn, however, can be distributed during grain harvest and transportation 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.

II.I. Characteristics of the Recipient Corn Material

The A x Hi-II germplasm was the recipient of the transgenes present in MON 88017. It was used because it responds well to transformation with *Agrobacterium* and tissue culture regeneration.

The Hi-II inbred germplasm was specifically developed for use in corn transformation (Armstrong et al., 1991) and is publicly available from the Maize Genetics Stock Center (USDA-ARS, 2003). The Hi-II germplasm was derived by crossing the publicly available Stiff Stalk inbreds, B73 and A188, with one another. Inbred B73 was released to the public in 1972, and is characterized by plants with upright leaves of a dark green color and ears of a larger than average size (Troyer, 1999). Inbred A is a private elite Stiff Stalk inbred that is commonly used as a female in breeding with other corn inbreds.

II.J. Corn as a Test System in this Petition

In developing the data in support of this petition, appropriate test and control materials were developed, and where feasible, conventional reference corn materials were used to establish a range of responses expected for commercial corn in the U.S. In general, the background of the test material was matched with that of the control material so that the effect of the genetic insert could be assessed in an unbiased manner. Where feasible, positive and negative hybrids (isolines) were used as test and control materials, respectively. Further descriptions of the test, control, and reference materials are provided in the methods and materials sections for each experiment.

III. Description of the Transformation System

MON 88017 was produced by *Agrobacterium*-mediated transformation of immature embryos of A x Hi-II corn tissue. *Agrobacterium tumefaciens* strain ABI, containing plasmid PV-ZMIR39 (Figure III-1), was the transformation vector. *Agrobacterium tumefaciens* strain ABI contains a disarmed Ti plasmid that is incapable of inducing tumor formation because of the deletion of the phytohormone genes originally present in the *Agrobacterium* Ti plasmid. PV-ZMIR39 contains both the left and right transfer-DNA (T-DNA) border sequences to facilitate transformation.

DNA was introduced into corn cells by *Agrobacterium*-mediated transformation of freshly isolated immature embryos. *Agrobacterium* containing plasmid PV-ZMIR39 was induced to be virulent by the use of acetosyringone. Ishida et al. (1996) and Rout and Armstrong (1997) have described the method of embryo isolation and inoculation using *Agrobacterium*. Following inoculation with *Agrobacterium*, the immature embryos were transferred to a co-culture medium for one to three days so that both the corn immature embyros and *Agrobacterium* were able to grow together such that the transformation of individual cells could occur. This process of *Agrobacterium*-mediated transformation of corn involves the attachment of the bacterium to the corn cells which leads to transfer of the region of DNA between the left and right borders of the binary plasmid (i.e., the T-DNA) into the corn genomic DNA.

Following this incubation period on the co-culture medium, the immature embryos were transferred to selection medium containing carbenicillin to eliminate *Agrobacterium*, and glyphosate to eliminate those cells that were not transformed, so that only cells containing the T-DNA survived. The resulting transformed cells were then subcultured several times on a selection medium and regenerated into plants according to the protocol described by Armstrong and Phillips (1988).

Plants were screened for insect resistance, glyphosate tolerance, and field performance. Of the many transformation events screened, MON 88017 was selected as the leading commercial candidate. Regulatory studies on MON 88017 were initiated to establish its substantial equivalence to conventional corn and to demonstrate the human health and environmental safety of the introduced CP4 EPSPS and Cry3Bb1 proteins. Figure III-2 displays a flow chart of the major steps involved in the transformation, selection, and development of MON 88017.

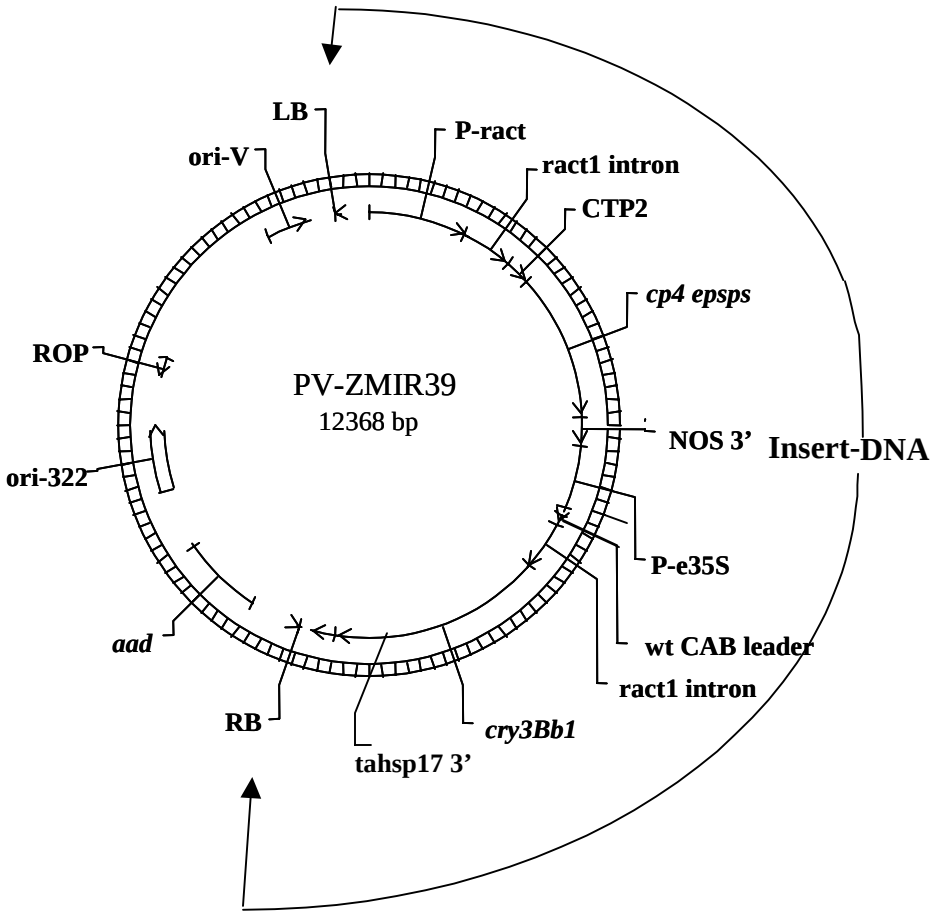


Figure III-1. Map of the Plasmid PV-ZMIR39

A circular map of the plasmid vector PV-ZMIR39 used in *Agrobacterium*-mediated transformation to create corn event MON 88017 is shown below. In this procedure, only the DNA present between the left and right borders (i.e., LB and RB) was transferred into the host corn cells.

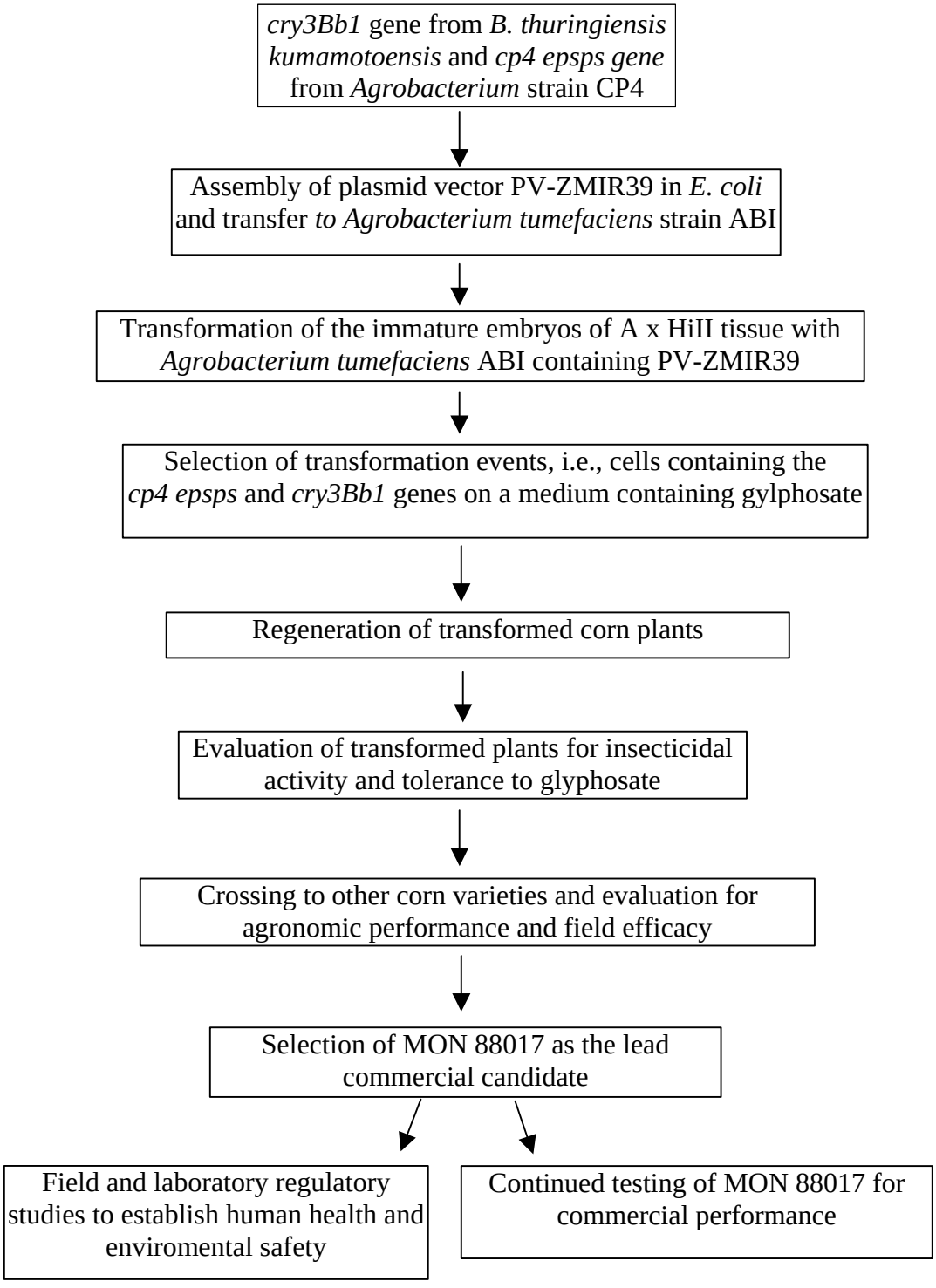


Figure III-2. Schematic of the Development of MON 88017

IV. Donor Genes and Regulatory Sequences

This section describes the donor genes and regulatory sequences used in the development of MON 88017 and the deduced amino acid sequences of the CP4 EPSPS and Cry3Bb1 proteins produced in MON 88017.

IV.A. Vector PV-ZMIR39

The plasmid vector PV-ZMIR39 (Figure III-1) was used for the transformation of corn cells to produce event MON 88017. It was constructed at Monsanto's research laboratories in St. Louis, Missouri, using standard molecular biology techniques. It is a disarmed, binary *Agrobacterium tumefaciens* transformation vector that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation. The inserted DNA region contains the *cp4 epsps* and *cryBb1* gene expression cassettes, and is the portion of plasmid PV-ZMIR39 that is integrated into the corn genome during the transformation process.

Starting from the left border, the inserted DNA contains: 1) the *cp4 epsps* coding sequence joined to a chloroplast transit peptide 2 (CTP2) sequence, regulated by the 5' noncoding end of the rice actin 1 sequence (ract 1) containing the promoter and first intron, and the nopaline synthase 3' polyadenylation sequence (NOS 3'); and 2) the *cry3Bb1* coding region regulated by the enhanced 35S plant promoter (e35S), a 5' untranslated leader of the wheat chlorophyll a/b/-binding protein (wt CAB leader), the ract1 intron, and the 3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 (tahsp17 3'), which ends transcription and provides the signal for mRNA polyadenylation. The specific genetic elements and origins of the various components used to construct plasmid vector PV-ZMIR39 are provided in Table IV-1.

IV.B. The *cp4 epsps* Coding Sequence and CP4 EPSPS Protein

The *cp4 epsps* coding sequence derived from *Agrobacterium sp.* strain CP4, a common soil-borne bacterium, has been sequenced and shown to encode a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). The CP4 EPSPS protein has been shown to provide high levels of tolerance to glyphosate when introduced into plants (Padgette, et al., 1996; OECD, 1999).[1] Glyphosate binds to and blocks the activity of the native EPSPS, an enzyme of the aromatic amino acid biosynthetic pathway. In plants, the EPSPS enzyme is located in the chloroplast. Thus, in the vector PV-ZMIR39, a CTP2 coding sequence was joined to the *cp4 epsps* coding sequence to target transport of the encoded protein to the chloroplast. The targeted CTP2-CP4 EPSPS protein contains 531 amino acids with a molecular weight of 55.8 kDa (Figure IV-1).

[1] See Section VI for further discussion on EPSPS biochemistry and the CP4 EPSPS protein.

IV.C. The *Arabidopsis thaliana* EPSPS Transit Peptide (CTP2)

In the plant gene expression cassette, the *cp4 epsps* coding sequence is joined to a chloroplast transit peptide 2 (CTP2) isolated from the *Arabidopsis thaliana epsps* gene (Klee et al., 1987). This transit peptide directs the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid biosynthesis (Kishore and Shah, 1988). Transit peptides are typically cleaved and degraded from the mature protein upon delivery to the plastid (Della-Cioppa et al., 1986).

IV.D. The *cp4 epsps* Regulatory Sequences

As noted above, the *ctp2-cp4 epsps* coding sequence is under the control of the ract1 sequence containing the promoter and first intron (McElroy et al., 1990) introduced upstream of the *ctp2* sequence. The *cp4 epsps* sequence is joined to the NOS 3' sequence from *Agrobacterium tumefaciens* (Fraley et al., 1983), that provides the transcription termination and the mRNA polyadenylation signal.

IV.E. The *cry3Bb1* Coding Sequence and Cry3Bb1 Protein

The *cry3Bb1* coding sequence from the wild-type *Bacillus thuringiensis* (subsp. *kumamotoensis*) strain EG4691 (Donovan et al., 1992), was modified to encode six specific amino acid substitutions (Figure IV-2), resulting in the synthetic *cry3Bb1* coding sequence present in plasmid vector PV-ZMIR39 (Romano, 2002). The Cry3Bb1 protein produced in MON 88017 is a member of the Cry3Bb class of proteins that share >95% amino sequence homology (Crickmore et al., 1998). It is a variant of the wild-type Cry3Bb1 protein with which it shares an amino acid sequence identity of 99.1%[2], differing by six of 652 amino acid residues. The amino acid sequences of the Cry3Bb1 variants present in MON 88017 and YieldGard Rootworm corn (MON 863) share an identity of 99.8% identity (they differ by only one of 653 amino acid residues). The Cry3Bb1 proteins in MON 863 and in MON 88017 have been extensively characterized.

IV.F. The Cry3Bb1 Regulatory Sequences

The synthetic *cry3Bb1* gene expression cassette that produces the Cry3Bb1 protein consists of the e35S promoter (Odell et al., 1985), the wt CAB leader (Lamppa et al., 1985), and the intron from the ract1 gene (McElroy et al., 1991) joined to the synthetic *cry3Bb1* coding sequence at the 5' end. Joined to the 3' end of the synthetic *cry3Bb1* coding sequence is the tahsp17 3' sequence, which ends transcription and provides the signal for mRNA polyadenylation (McElwain and Spiker, 1989).

[2] The wild-type Cry3Bb1 protein consists of 652 amino acid residues (Donovan et al., 1992). In MON 88017 the Cry3Bb1 protein (653 amino acid residues) contains an additional amino acid (alanine) at position 2, because it was necessary to create an *Nco* I restriction endonuclease site for the development of the plant gene expression plasmid vector PV-ZMIR39.

IV.G. T-DNA Borders

Plasmid vector PV-ZMIR39 contains DNA sequences that are necessary for transfer of T-DNA into the plant cell. These sequences are termed the Right and Left Border regions and they define the extent of the DNA that should be transferred into the plant genome. The Left Border is a 25 bp nucleotide sequence, contained within a 34 bp region, isolated from *A. tumifaciens* plasmid pTi5955, a derivative of plasmid pTiA6 (Barker et. al., 1983). The Right Border is a 24 bp nucleotide sequence that was originally isolated from *A. tumifaciens* plasmid pTiT37 (Depicker et al., 1982).

IV.H. Genetic Elements Outside the T-DNA Borders

The backbone region outside of the inserted DNA, which is not integrated into the corn genome during transformation (see Section V), contains a bacterial selectable marker gene, *aad*, which encodes an aminoglycoside-modifying enzyme that is resistant to the action of the antibiotics spectinomycin and streptomycin, as well as two origins of replication necessary for replication and maintenance of the plasmid in bacteria. A detailed description of all elements in the bacterial backbone region is presented in Table IV-1.

Table IV-1. Summary of the Genetic Elements in PV-ZMIR39

Genetic elements in the cp4 epsps expression cassette				
Genetic Element	**Location in plasmid (bp)**	**Donor**	**Function**	**Bibliographic reference**
LB (Left Border)	12067-12090	Octopine Ti plasmid, pTi15955	Left border sequence essential for transfer of T-DNA from the octopine Ti plasmid, pTi15955	Barker et al., 1983.
Intervening sequence	12091-12364	*Agrobacterium*	Polylinker	Barker et al., 1983.
Intervening sequence	12365-12	Synthetic	Polylinker	
P-ract1	13 - 946	Rice actin gene	Promoter	McElroy et al., 1990.
ract1 intron	947 - 1407	Rice actin gene	Intron	McElroy et al., 1991.
Intervening sequence	1408-1423	Synthetic	Polylinker	
CTP2	1424 -1651	*Arabidopsis thaliana*	Chloroplast transit peptide sequence	Klee et al., 1987.
cp4 epsps	1652-3019	*Agrobacterium sp*. strain CP4	Coding sequence for the native CP4 EPSPS protein	Padgette et al., 1996.
Intervening sequence	3020-3031	Synthetic	Polylinker	
NOS 3'	3032-3287	*Agrobacterium tumefaciens*	3'nontranslated region of the nopaline synthase (NOS) coding sequence, which terminates transcription and directs polyadenylation	Bevan et al., 1983.
Intervening sequence	3288-3320	Synthetic	Polylinker	

Table IV-1 (Continued). Summary of the Genetic Elements in PV-ZMIR39

Genetic elements in the *cry3Bb1* expression cassette				
Genetic Element	**Location in plasmid (bp)**	**Donor**	**Function**	**Bibliographic reference**
P-e35S	3321-3933	Cauliflower mosaic virus	Promoter with the duplicated enhancer region	Odell et al., 1985. Kay et al., 1987.
Intervening sequence	3934-3957	Synthetic	Polylinker	
wt CAB leader	3958-4028	Wheat	5' untranslated leader of the wheat chlorophyll a/b-binding protein	Lamppa et al., 1985.
Intervening sequence	4029-4056	Synthetic	Polylinker	
ract1 intron	4057-4517	Rice actin gene	Intron	McElroy et al., 1991.
Intervening sequence	4518-4533	Synthetic	Polylinker	
cry3Bb1	4534-6495	*Bacillus thuringiensis* subsp. *kumamotoensis*	Coding sequence for a synthetic variant of Cry3Bb1 protein	Romano, 2002.
Intervening sequence	6496-6510	Synthetic	Polylinker	
tahsp17 3'	6511-6744	Wheat heat shock protein	3'nontranslated region of the coding sequence for wheat 17.3 kDa heat-shock protein, which ends transcription and directs polyadenylation	McElwain and Spiker, 1989.
Intervening sequence	6745-6840	*E. coli* and synthetic	Polylinker	Depicker et al., 1982.
RB (Right Border)	6841-6865	Nopaline Ti plasmid, pTiT37	Right border sequence essential for transfer of T-DNA from the nopaline Ti plasmid, pTiT37	Depicker et al., 1982.

Table IV-1 (Continued). Summary of the Genetic Elements in PV-ZMIR39

Backbone genetic elements for expression of the plasmid in *E. coli*				
Genetic Element	**Location in Plasmid (bp)**	**Donor**	**Function**	**Bibliographic reference**
Intervening sequence	6866-7350	*E. coli* and synthetic	Polylinker	Depicker et al., 1982. Sutcliffe, 1978. Fling et al., 1985.
aad	7351-8139	Bacterial transposon Tn7	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7	Fling et al., 1985. GenBank accession X03043
Intervening sequence	8140-8681	*E. coli*	Polylinker	Fling et al., 1985. Sutcliffe, 1978.
ori-322	8682-9310	Plasmid pBR322 from *E. coli*	Origin of replication from pBR322 for maintenance of plasmid in *E. coli*	Sutcliffe, 1978.
Intervening sequence	9311-9727	Plasmid pBR322 from *E. coli*	Portion of the plasmid	Sutcliffe, 1978.
ROP	9728-9919	*E. coli*	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*	Giza and Huang, 1989.
Intervening sequence	9920-11182	Plasmid pBR322 from *E. coli*	Portion of the plasmid	Sutcliffe, 1978.
Intervening sequence	11183-11430	*E. coli* and synthetic	Plasmid DNA	Stalker et al., 1981.
ori-V	11431-11824	*Agrobacterium*, plasmid RK2	Origin of replication for *Agrobacterium*	Stalker et al., 1981.
Intervening sequence	11825-11910	*E. coli* and synthetic	Plasmid DNA	Stalker et al., 1981.
Intervening sequence	11911-12066	*Agrobacterium*	DNA sequences	Barker et al., 1983.

```
  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-1. Deduced Amino Acid Sequence for the CP4 EPSPS Protein Produced in MON 88017

Sequence includes the CTP2 transit peptide (amino acids 1-76 underlined).

```
  1  M**A**NPNNRSEH DTIKVTPNSE LQTNHNQYPL ADNPNSTLEE LNYKEFLRMT
 51  EDSSTEVLDN STVKDAVGTG ISVVGQILGV VGVPFAGALT SFYQSFLNTI
101  WPSDADPWKA FMAQVEVLID KKIEEYAKSK ALAELQGLQN NFEDYVNALN
151  SWKKTPLSLR SKRSQDRIRE LFSQAESHFR NSMPSFAVSK FEVLFLPTYA
201  QAANTHLLLL KDAQVFGEEW GYSSEDVAEF Y**R**RQLKLTQQ YTDHCVNWYN
251  VGLNGLRGST YDAWVKFNRF RREMTLTVLD LIVLFPFYDI RLYSKGVKTE
301  LTRDIFTDPI F**L**L**T**TLQ**K**YG PTFLSIENSI RKPHLFDYLQ GIEFHTRL**R**P
351  GYFGKDSFNY WSGNYVETRP SIGSSKTITS PFYGDKSTEP VQKLSFDGQK
401  VYRTIANTDV AAWPNGKVYL GVTKVDFSQY DDQKNETSTQ TYDSKRNNGH
451  VSAQDSIDQL PPETTDEPLE KAYSHQLNYA ECFLMQDRRG TIPFFTWTHR
501  SVDFFNTIDA EKITQLPVVK AYALSSGASI IEGPGFTGGN LLFLKESSNS
551  IAKFKVTLNS AALLQRYRVR IRYASTTNLR LFVQNSNNDF LVIYINKTMN
601  KDDDLTYQTF DLATTNSNMG FSGDKNELII GAESFVSNEK IYIDKIEFIP
651  VQL
```

Figure IV-2. Deduced Amino Acid Sequence of the Cry3Bb1 Protein Produced in MON 88017

The six amino acids that differ from the wild-type Cry3Bb1 protein are indicated in underlined bold. The differences at the six positions are: 2A (insertion), H232R, S312L, N314T, E318K, Q349R.

V. Genetic Analysis

Molecular analysis was performed to characterize the DNA insert in MON 88017. This analysis demonstrated that MON 88017 contains a single, intact insert comprised of two expression cassettes: 1) the *cp4 epsps* coding sequence joined to a CTP2 sequence, regulated by the ract1 promoter and intron, and the NOS 3' polyadenylation sequence; and, 2) the *cry3Bb1* coding sequence regulated by the e35S plant promoter, the wt CAB leader, the ract1 intron, and the tahsp17 3' sequence which ends transcription and provides the signal for mRNA polyadenylation.

The data also show that no backbone sequences or additional elements from the transformation vector PV-ZMIR39, linked or unlinked to intact gene cassettes, were present in the genome of MON 88017. Insert stability analysis demonstrated that the expected Southern blot fingerprint of MON 88017 was maintained across the branches of the breeding tree that were tested, thereby confirming the stability of the insert over multiple generations. Finally, polymerase chain reaction (PCR) and DNA sequence analyses were performed to determine the 5' and 3' insert-to-plant junctions, and to confirm the organization of the elements within the insert. The results of the molecular analysis support the conclusion that only the expected full-length CP4 EPSPS and Cry3Bb1 proteins are encoded by the DNA insert present in MON 88017.

The following sections describe the molecular analysis experiments that were performed to characterize the integrated DNA in MON 88017. Southern blot analysis was used to assess the following:

1) number of insertions of the integrated expression cassettes,
2) number of copies of the integrated expression cassettes,
3) intactness of both expression cassettes,
4) the presence or absence of plasmid backbone sequences, and
5) the stability of the inserted DNA during conventional breeding.

Detailed materials and methods for the molecular characterization are provided in Appendix A. A plasmid map illustrating the location of each element in PV-ZMIR39 is presented in Figure III-1. Restriction enzyme sites and probes used for Southern blot analysis are presented in Figures V-1a and V-1b. A schematic presentation of the DNA inserted into the genome of MON 88017, including restriction enzyme sites and expected restriction fragments is provided in Figure V-2.

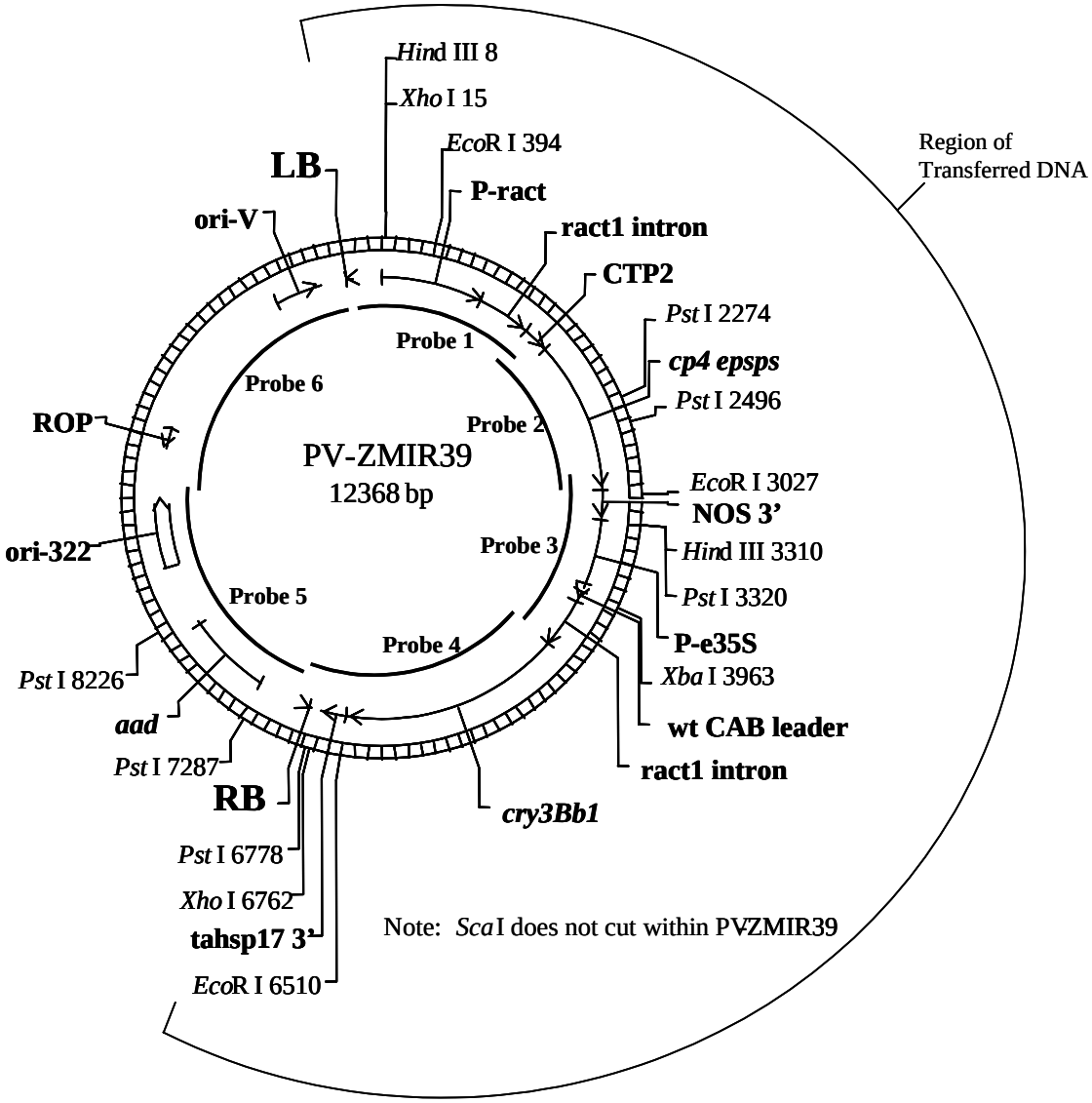


Figure V-1a. Restriction Enzymes and Probes 1-6 Generated from Vector PV-ZMIR39 for Southern Blot Analyses

Genetic elements are annotated in bold and restriction sites (with positions relative to the size of the plasmid vector) are shown for enzymes used in the Southern analyses. DNA probes were prepared by PCR amplification of plasmid PV-ZMIR39. The table describes probes 1-6 used in the Southern analyses.

Probe	Genetic Element(s)	Start	End	Total Length (bp)
1	Partial LB + P-ract + partial CTP2	12080	1482	1771
2	CTP2 + *cp4 epsps*	1423	3019	1597
3	Partial *cp4 epsps* + NOS 3' + P-e35S + wt CAB leader + ract1 intron	2991	4533	1543
4	*cry3Bb1* + tahsp17 3' + RB	4534	6865	2332
5	Partial backbone	6866	9492	2627
6	Partial backbone	9465	12066	2602

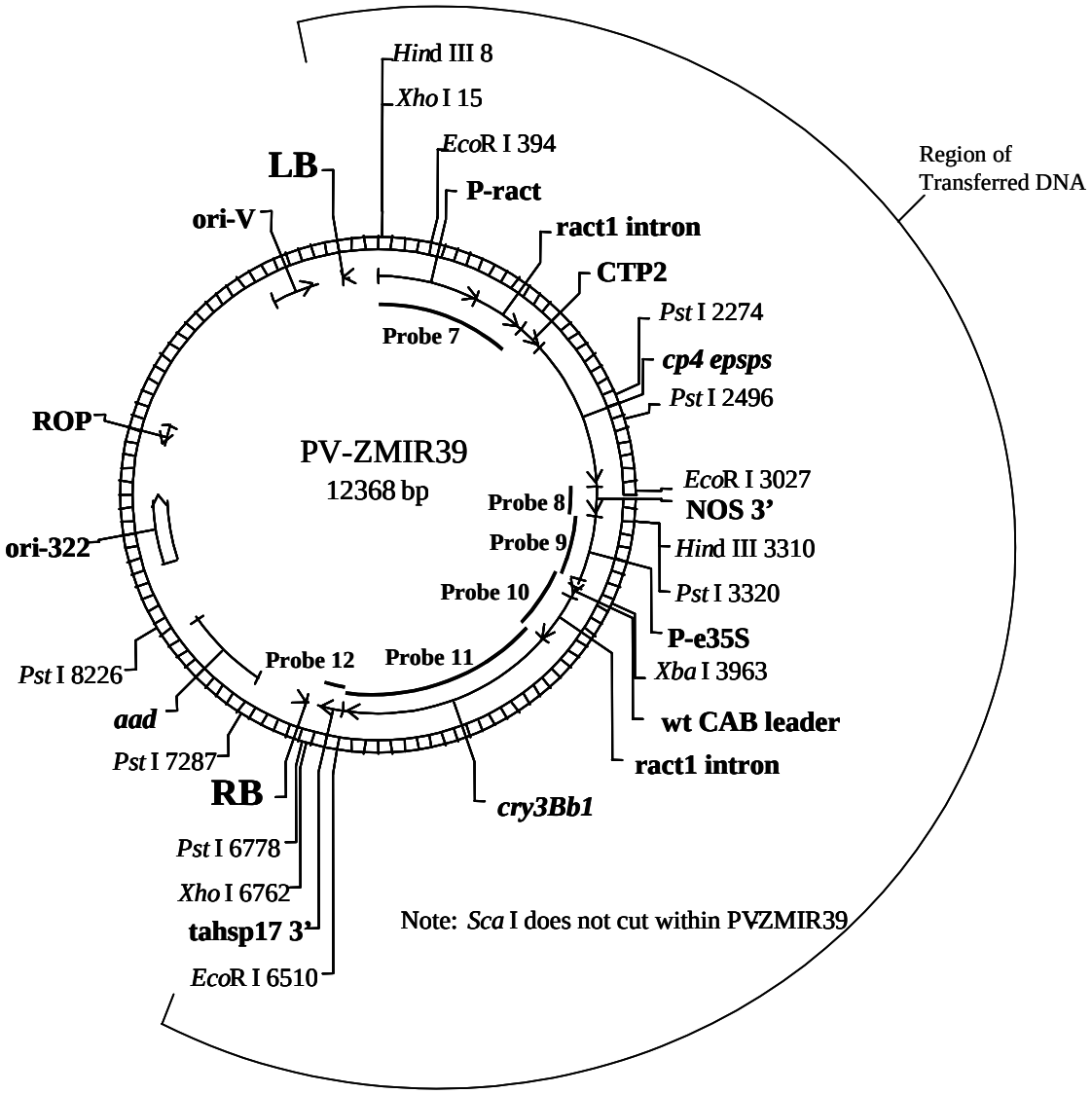


Figure V-1b. Restriction Enzymes and Probes 7-12 Generated from Vector PV-ZMIR39 for Southern Blot Analyses

Genetic elements are annotated in bold and restriction sites (with positions relative to the size of the plasmid vector) are shown for enzymes used in the Southern analyses. DNA probes were prepared by PCR amplification of plasmid PV-ZMIR39. The table describes probes 7-12 used in the Southern analyses.

Probe	Genetic Element(s)	Start	End	Total Length (bp)
7	P-ract1 + ract1 intron	13	1407	1395
8	NOS 3'	3032	3287	256
9	P-e35S	3321	3933	613
10	wt CAB leader + ract1 intron	3958	4533	576
11	*cry3Bb1*	4534	6510	1977
12	tahsp17 3'	6511	6744	234

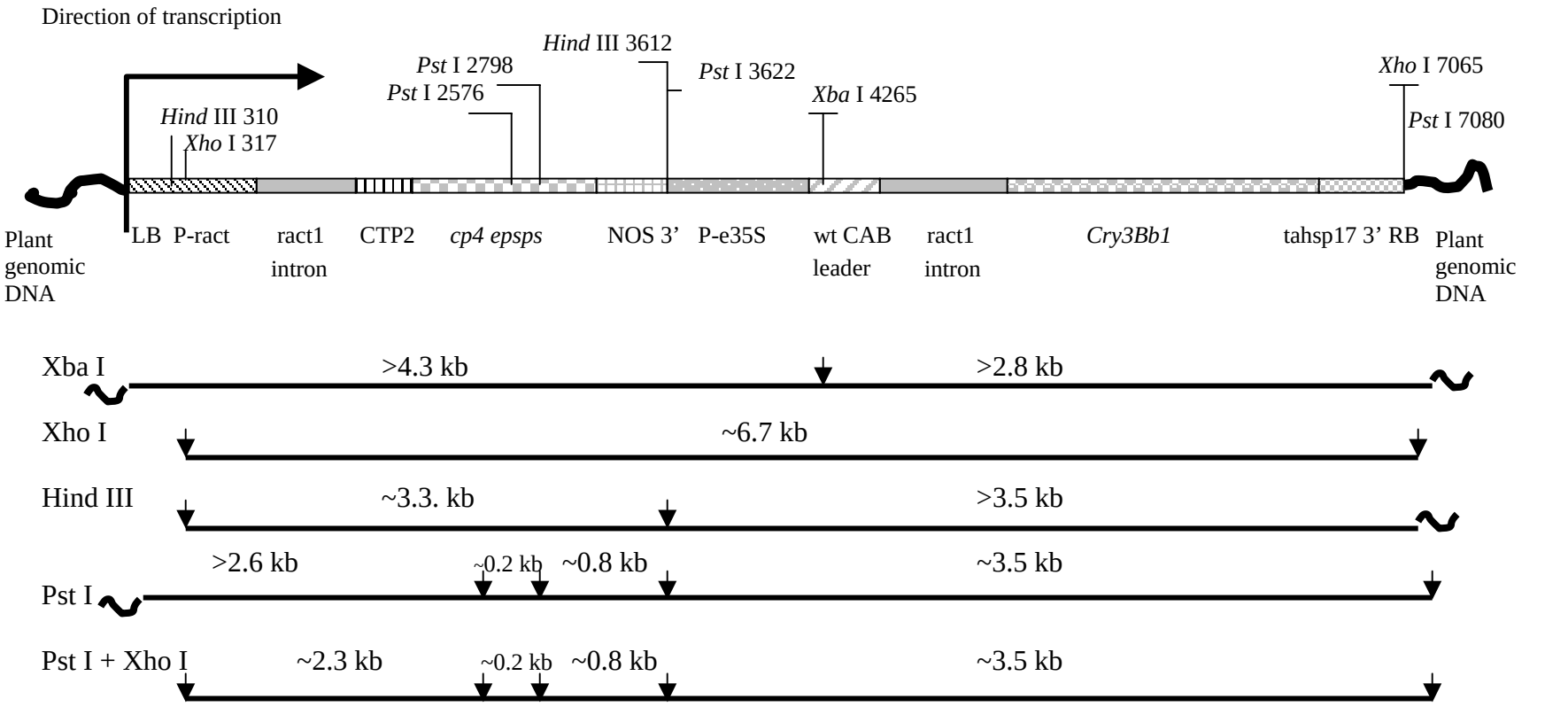


Figure V-2. Schematic Representation of the Insert Present in MON 88017

A linear map of the inserted DNA from transformation vector PV-ZMIR39 is shown. Genetic elements are annotated. Positions of the restriction sites for enzymes used in the Southern blot analyses are included for reference. Arrows indicate sites of the restriction digest. Sizes of predicted restriction fragments, calculated from the size of the linear map are identified. MON 88017 contains one copy of the insert at a single integration locus.

V.A. Insert and Copy Number

The insert number (the number of integration sites of the introduced DNA in the corn genome) was evaluated by digesting the MON 88017 and conventional corn genomic DNA (control in Figure V-3) with the restriction enzyme *Sca* I, which does not cleave within the plasmid PV-ZMIR39. This enzyme should release a restriction fragment containing the inserted DNA and adjacent plant genomic DNA. The number of restriction fragments detected indicates the number of inserts present.

The number of copies of the introduced DNA was determined by digesting the MON 88017 genomic DNA with *Xba* I, a restriction enzyme that cuts only once within PV-ZMIR39. The blot was probed simultaneously with four radiolabeled fragments of plasmid DNA that span the entire length of the insert (Probes 1, 2, 3 and 4, Figure V-1a). If one copy of the introduced DNA is present, two bands should be produced, representing two border fragments. Each of these border fragments would contain a portion of the inserted DNA and flanking corn genomic DNA. Therefore, from this analysis it was possible to determine the number of copies of the introduced DNA inserted in MON 88017. Plasmid PV-ZMIR39 DNA, previously digested with *Eco*R I, was mixed with control corn genomic DNA digested with *Sca* I, and then loaded on the gel to serve as a positive hybridization control.

The results of these analyses are shown in Figure V-3. Plasmid PV-ZMIR39 DNA, previously digested with *Eco*R I, mixed with control DNA digested with *Sca* I, produced the expected size bands of approximately 6.3 kb (faint), 3.5 kb and 2.6 kb, correlating to the sizes of PV-ZMIR39 *Eco*R I fragments (lanes 4 and 5). The ~6.3 kb band produced a weaker signal, because a smaller portion of the target DNA sequence is present on this ~6.3 kb *Eco*R I restriction fragment in comparison to the ~3.5 kb and ~2.6 kb fragments. MON 88017 genomic DNA digested with *Sca* I (lanes 2 and 6) produced one band at approximately 13 Kb. This result establishes that MON 88017 contains one insert located on an approximately 13 kb *Sca* I restriction fragment. MON 88017 DNA digested with *Xba* I (lanes 3 and 7) produced two unique bands at approximately 7.4 kb and 5.5 kb, representing two border fragments. In combination with the insert number analysis, this result establishes that MON 88017 contains one copy of the introduced DNA at a single locus of integration.

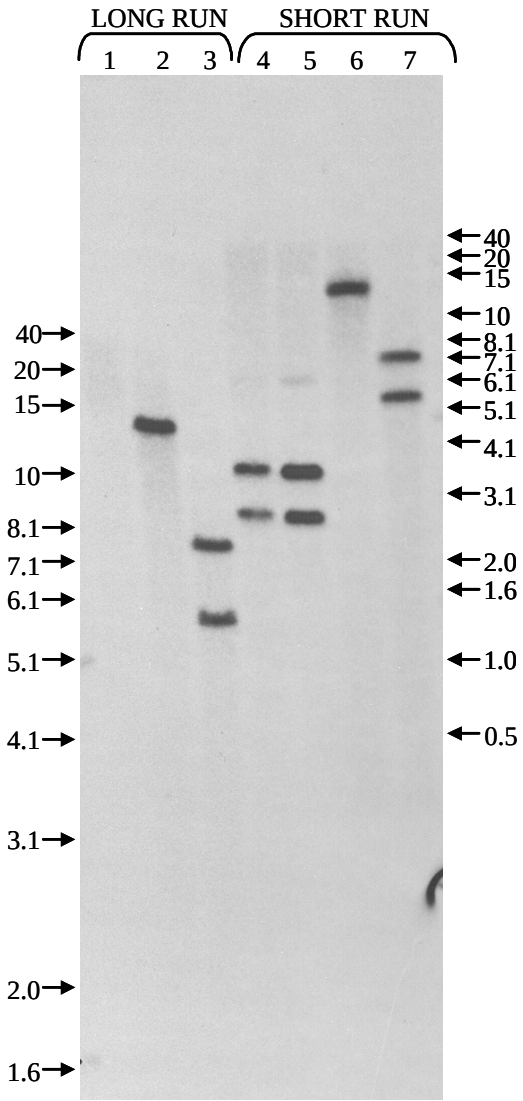


Figure V-3. Southern Blot Analysis of MON 88017: Insert and Copy Number

The blot was probed simultaneously with four ^{32}P-labeled probes that span the full length of the insert (Probes 1, 2, 3, and 4, Figure V-1a). Each lane contains ~10 μg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Sca* I)
2: MON 88017 (*Sca* I)
3: MON 88017 (*Xba* I)
4: Control (*Sca* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
5: Control (*Sca* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
6: MON 88017 (*Sca* I)
7: MON 88017 (*Xba* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

V.B. *cp4 epsps* Cassette Intactness

MON 88017 corn genomic DNA was digested individually with *Xho* I to release the inserted DNA and with *Hin*d III to release the *cp4 epsps* cassette. Control genomic DNA from conventional corn was digested with *Xho* I and/or with *Hin*d III, to serve as a negative control. Plasmid PV-ZMIR39 DNA, previously digested with *Eco*R I, was mixed with control DNA digested with *Xho* I, and the mixture was then loaded on the gel to serve as a positive hybridization control. Individual Southern blots were probed with the rice actin promoter (P-ract) + ract1 intron, the CTP2 + *cp4 epsps* coding region, or the NOS 3' polyadenylation sequence (Probes 7, 2, and 8, respectively, Figures V-1a, b). The presence of a band representing the expected size of the full inserted DNA (*Xho* I digest, ~6.7 kb) or the *cp4 epsps* cassette (*Hin*d III digest, ~3.3 kb) indicates that the *cp4 epsps* cassette and each of its elements are intact. For reference, the expected sizes of the full insert and *cp4 epsps* cassette upon restriction enzyme digestion are shown in Figure V-3. In these Southern analyses, the migration of the ~6.7 kb *Xho* I fragment containing the entire insert was slightly slower than expected. This altered migration is likely because of the difference in salt concentrations between the test DNA sample and the molecular weight marker (Sambrook and Russell, 2001).

V.B.1. P-ract1 + Ract1 Intron Probe

The results of this analysis are shown in Figure V-4. The Southern blot containing control, plasmid and MON 88017 genomic DNA digested with *Xho I* or *Hind III* was hybridized with the P-ract1 + ract intron probe (Probe 7, Figure V-1b). Control DNA digested with *Xho* I or *Hin*d III (lanes 1 and 2, respectively) showed faint endogenous hybridization, indicating regions of homology between the corn genome and the probe. Plasmid PV-ZMIR39 DNA, previously digested with *Eco*R I, mixed with control genomic DNA digested with *Xho* I, produced the expected size bands at approximately 3.5 kb and 2.6 kb (lanes 5 and 6). MON 88017 DNA digested with *Xho* I (lanes 3 and 7) produced a band corresponding to the expected size of the insert (approximately 6.7 kb) in addition to the faint endogenous hybridization pattern seen in the negative control (lane 1). MON 88017 DNA digested with *Hin*d III (lanes 4 and 8) produced bands at approximately 8.5 kb and 3.3 kb in addition to the faint endogenous hybridization pattern seen in the control (lane 2). The ~3.3 kb band corresponds to the expected size of an intact *cp4 epsps* cassette. The ~8.5 kb band corresponds to a border fragment predicted by hybridization of the ract1 intron portion of the probe to the ract1 intron target sequence present in the *cry3Bb1* cassette. No unexplained bands were detected, indicating that MON 88017 does not contain any additional, detectable rice actin promoter or ract1 intron elements other than those associated with the intact *cp4 epsps* or *cry3Bb1* cassettes.

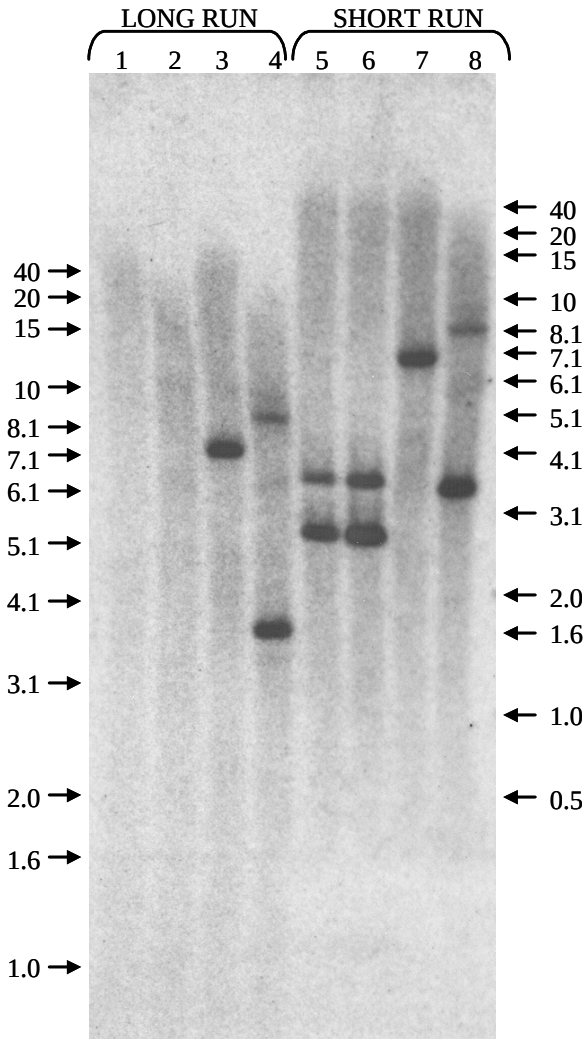


Figure V-4. Southern Blot Analysis of MON 88017: P-ract1 + Ract1 Intron Probe

The blot was probed with ^{32}P-labeled rice actin promoter and intron (Probe 7, Figure V-1b). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Xho* I)
2: Control (*Hin*d III)
3: MON 88017 (*Xho* I)
4: MON 88017 (*Hin*d III)
5: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
6: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
7: MON 88017 (*Xho* I)
8: MON 88017 (*Hin*d III)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

V.B.2. CTP2 + *cp4 epsps* Probe

The results of this analysis are shown in Figure V-5. The Southern blot containing control, plasmid and MON 88017 genomic DNA digested with *Xho I* or *Hind III* was hybridized with CTP2 + *cp4 epsps* probe (Probe 2, Figure V-1a). Control DNA digested with *Xho* I or *Hin*d III (lanes 1 and 2, respectively) showed no detectable hybridization bands, as expected for a negative control. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I, mixed with control DNA digested with *Xho* I, produced the expected size band at approximately 2.6 kb (lanes 5 and 6). MON 88017 genomic DNA digested with *Xho* I (lanes 3 and 8) produced a band corresponding to the expected size of the insert (approximately 6.7 kb). MON 88017 DNA digested with *Hin*d III (lanes 4 and 7) produced a band at approximately 3.3 kb, corresponding to the expected size of an intact *cp4 epsps* cassette. No unexpected bands were detected, indicating that MON 88017 does not contain any additional, detectable CTP2 + *cp4 epsps* coding regions other than that associated with the intact *cp4 epsps* cassette.

V.B.3. NOS 3' Probe

The results of this analysis are shown in Figure V-6. The Southern blot containing control, plasmid and MON 88017 genomic DNA digested with *Xho I* or *Hind III* was hybridized with the NOS 3' probe (Probe 8, Figure V-1b). Control DNA digested with *Xho* I (lane 1) showed no detectable hybridization bands, as expected for a negative control. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I, mixed with control DNA digested with *Xho* I, produced the expected size band at approximately 3.5 kb (lanes 4 and 5). MON 88017 DNA digested with *Xho* I (lanes 2 and 6) produced a band corresponding to the expected size of the insert (approximately 6.7 kb). MON 88017 DNA digested with *Hin*d III (lanes 3 and 7) produced a band at approximately 3.3 kb, corresponding to the expected size of an intact *cp4 epsps* cassette. No unexpected bands were detected, indicating that MON 88017 does not contain any additional, detectable NOS 3' polyadenylation elements other than that associated with the intact *cp4 epsps* cassette.

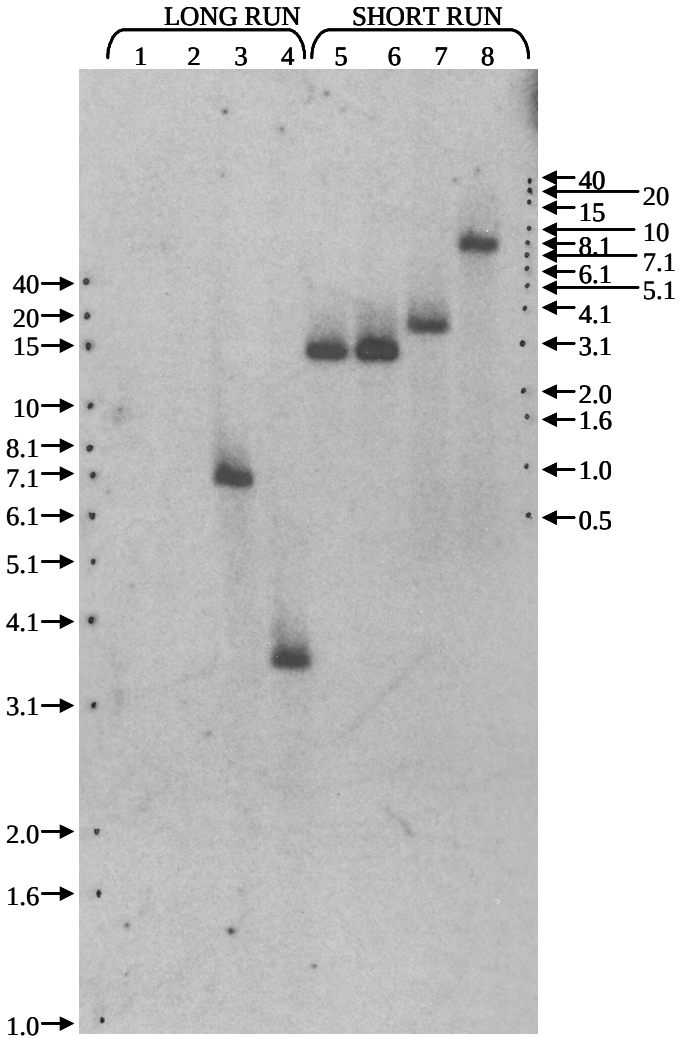


Figure V-5. Southern Blot Analysis of MON 88017: CTP2 + *cp4 epsps* Probe

The blot was probed with ^{32}P-labeled CTP2 + *cp4 epsps* (Probe 2, Figure V-1a). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Xho* I)
2: Control (*Hin*d III)
3: MON 88017 (*Xho* I)
4: MON 88017 (*Hin*d III)
5: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
6: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
7: MON 88017 (*Hin*d III)
8: MON 88017 (*Xho* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

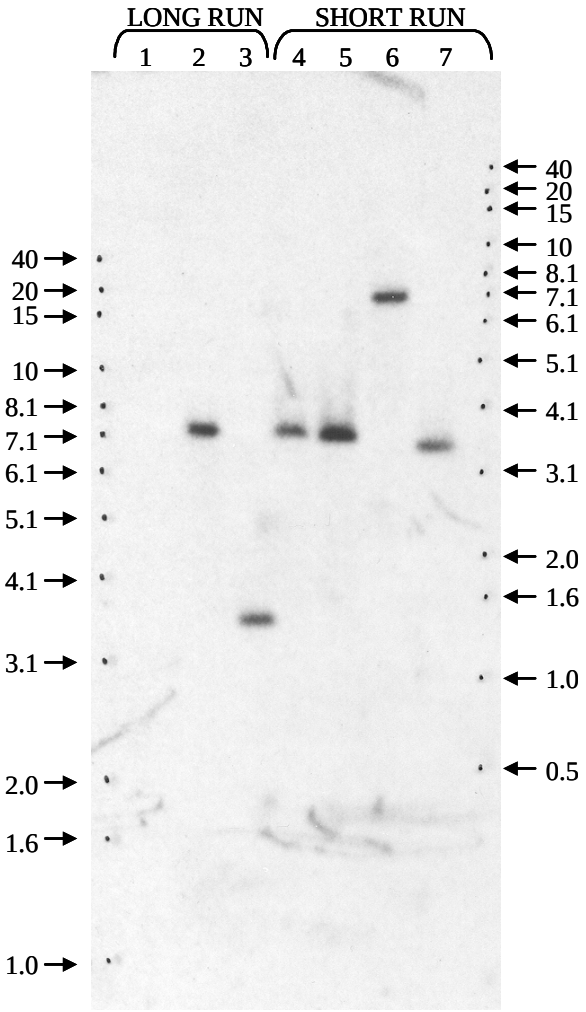


Figure V-6. Southern Blot Analysis of MON 88017: NOS 3' Probe

The blot was probed with ^{32}P-labeled NOS 3' polyadenylation sequence (Probe 8, Figure V-1b). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Xho* I)
2: MON 88017 (*Xho* I)
3: MON 88017 (*Hin*d III)
4: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
5: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
6: MON 88017 (*Xho* I)
7: MON 88017 (*Hin*d III)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

V.C. *cry3Bb1* Cassette Intactness

MON 88017 genomic DNA was digested with *Xho* I to release the inserted DNA. Additionally, MON 88017 genomic DNA was digested with *Pst* I to release the *cry3Bb1* cassette. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I, was mixed with control DNA digested with *Xho* I, and the mixture was then loaded on the gel to serve as a positive hybridization control. Control genomic DNA was digested with *Xho* I to serve as a negative control. Individual Southern blots were hybridized with the enhanced 35S promoter (P-e35S), the wt CAB leader + ract1 intron, the *cry3Bb1* coding region, or the tahsp17 3' polyadenylation sequence (Probes 9, 10, 11 and 12, respectively, Figure V-1b). The presence of a band representing the expected size of the full insert (*Xho* I digest, ~6.7 kb) or the *cry3Bb1* cassette (*Pst* I digest, ~3.5 kb) indicates that the *cry3Bb1* cassette and each of its elements are intact. For reference, the expected sizes of the full insert and the *cry3Bb1* cassette upon restriction enzyme digestion are shown in Figure V-2. Similar to the *cp4 epsps* Southern analyses, occasionally the migration of the ~6.7 kb *Xho* I fragment containing the entire insert was slightly slower than expected. This altered migration is likely because of the difference in salt concentrations between the test DNA sample and the molecular weight marker (Sambrook and Russell, 2001).

V.C.1. P-e35S Probe

The results of this analysis are shown in Figure V-7. The Southern blot containing control, plasmid and MON 88017 genomic DNA digested with *XhoI* or *PstI* was hybridized with the P-e35S probe (Probe 9, Figure V-1b). Control genomic DNA digested with *Xho* I (lane 1) showed no detectable hybridization bands, as expected for the negative control. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I, mixed with control DNA digested with *Xho* I, produced the expected size band at approximately 3.5 kb (lanes 4 and 5). MON 88017 genomic DNA was digested with *Xho* I (lanes 2 and 6) produced a band corresponding to the expected size of the inserted DNA (approximately 6.7 kb). MON 88017 genomic DNA digested with *Pst* I (lanes 3 and 7) produced a band at approximately 3.5 kb, corresponding to the expected size of an intact *cry3Bb1* cassette. No unexpected bands were detected, indicating that MON 88017 does not contain any additional, detectable enhanced 35S promoter elements other than that associated with the intact *cry3Bb1* cassette.

V.C.2. wt CAB Leader + Ract1 Intron Probe

The results of this analysis are shown in Figure V-8. The Southern blot containing control, plasmid and MON 88017 genomic DNA digested with *Xho I* or *Pst I* was hybridized with the wt CAB leader + ract1 intron probe (Probe 10, Figure V-1b). Control genomic DNA digested with *Xho* I (lane 1) showed no detectable hybridization bands, as expected for a negative control. Plasmid PV-ZMIR39 DNA, previously digested with *Eco*R I, mixed with control DNA digested with *Xho* I, produced the expected size bands at approximately 3.5 kb and 2.6 kb (lanes 4 and 5). MON 88017 genomic DNA digested with *Xho* I (lanes 2 and 7) produced a band corresponding to the expected size of the insert (approximately 6.7 kb). MON 88017 genomic DNA digested with *Pst* I (lanes 3 and 6) produced bands at approximately 3.8 kb and 3.5 kb. The ~3.5 kb band corresponds to the expected size of the intact *cry3Bb1* cassette. The ~3.8 kb band

corresponds to a border fragment predicted by hybridization of the ract1 intron portion of the probe to the ract1 intron target sequence present in the *cp4 epsps* cassette. No unexpected bands were detected, indicating that MON 88017 does not contain any additional, detectable wt CAB leader or ract1 intron elements other than those associated with the intact *cry3Bb1* or *cp4 epsps* cassettes.

V.C.3. *cry3Bb1* Probe

The results of this analysis are shown in Figure V-9. The Southern blot containing control, plasmid and MON 88017 genomic DNA digested with *Xho I* or *Hind III* was hybridized with the *cry3Bb1* probe (Probe 11, Figure V-1b). Control genomic DNA digested with *Xho* I (lane 1) showed no detectable hybridization bands, as expected for the negative control. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I, mixed with control DNA digested with *Xho* I, produced the expected size band at approximately 3.5 kb (lanes 4 and 5). MON 88017 genomic DNA digested with *Xho* I (lanes 2 and 6) produced a band corresponding to the expected size of the insert (approximately 6.7 kb). MON 88017 genomic DNA digested with *Pst* I (lanes 3 and 7) produced a band at approximately 3.5 kb, corresponding to the expected size of an intact *cry3Bb1* cassette. No unexpected bands were detected, indicating that MON 88017 does not contain any additional, detectable *cry3Bb1* coding regions other than that associated with the intact *cry3Bb1* cassette.

V.C.4. tahsp17 3' Probe

The results of this analysis are shown in Figure V-10. The Southern blot containing control, plasmid and MON 88017 genomic DNA digested with *Xho* I or *Pst* I was hybridized with the tahsp17 3' probe (Probe 12, Figure V-1b). Control corn genomic DNA digested with *Xho* I (lane 1) showed no detectable hybridization bands, as expected for a negative control. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I, mixed with control genomic DNA digested with *Xho* I, produced the expected size band at approximately 6.3 kb (lanes 4 and 5). MON 88017 genomic DNA digested with *Xho* I (lanes 2 and 6) produced a band corresponding to the expected size of the insert (approximately 6.7 kb). MON 88017 genomic DNA digested with *Pst* I (lanes 3 and 7) produced a band at approximately 3.5 kb, corresponding to the expected size of an intact *cry3Bb1* cassette. A small area of hybridization was present in lane 3 directly above the expected hybridization signal. This spot does not span the entire lane, and does not appear in the short run of the same sample in lane 7, indicating that this signal is an artifact. No unexpected bands were detected, indicating that MON 88017 does not contain any additional, detectable tahsp17 3' polyadenylation elements other than that associated with the intact *cry3Bb1* cassette.

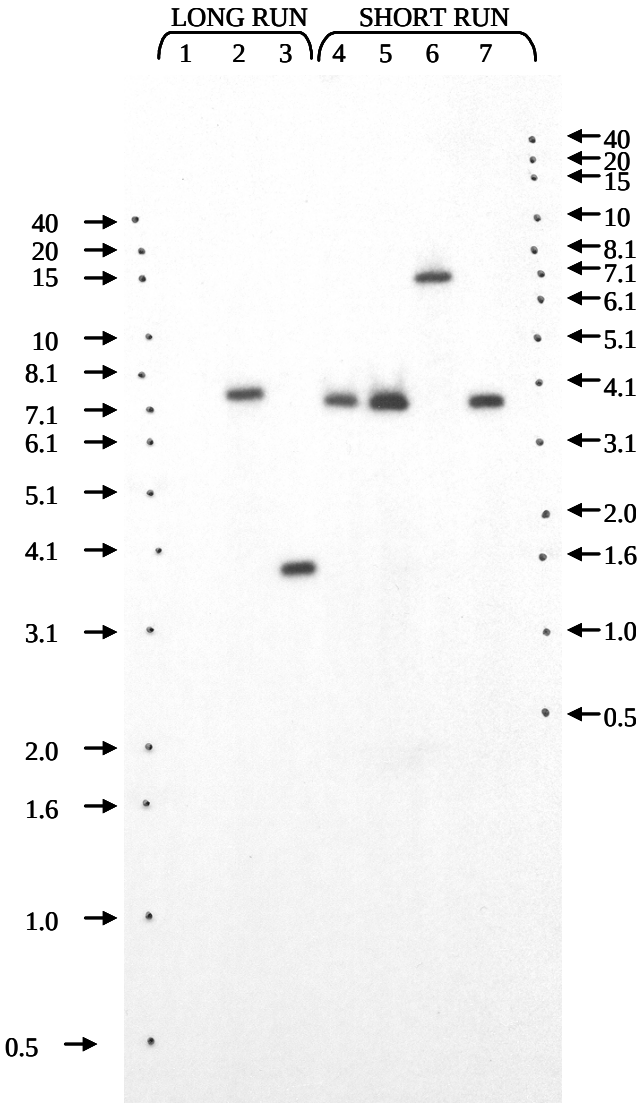


Figure V-7. Southern Blot Analysis of MON 88017: P-e35S Probe

The blot was probed with ^{32}P-labeled enhanced 35S promoter (Probe 9, Figure V-1b). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Xho* I)
2: MON 88017 (*Xho* I)
3: MON 88017 (*Pst* I)
4: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
5: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
6: MON 88017 (*Xho* I)
7: MON 88017 (*Pst* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

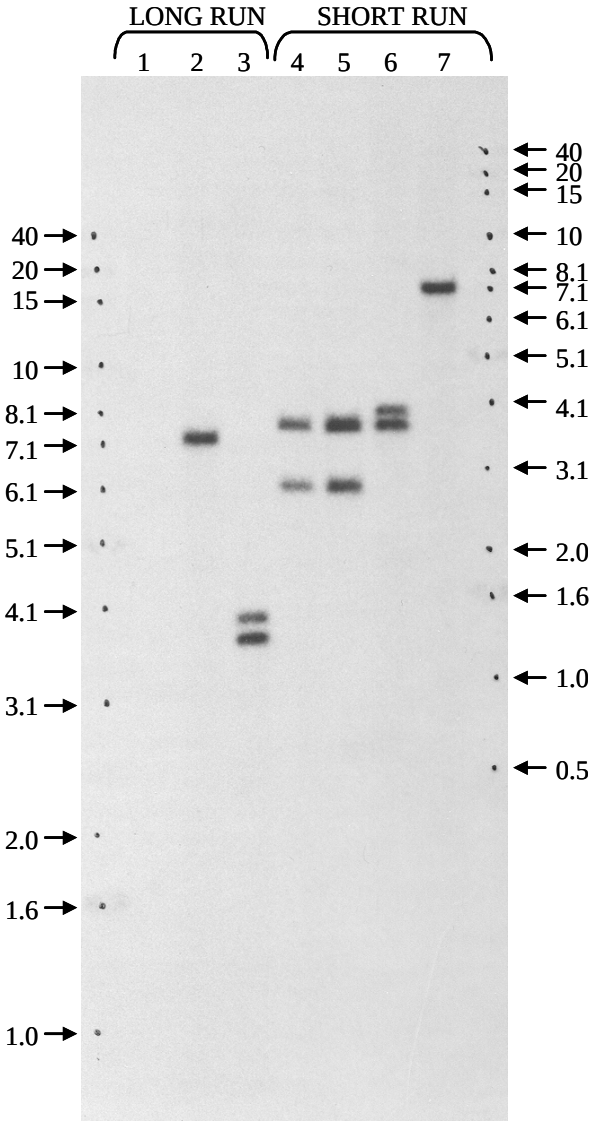


Figure V-8. Southern Blot Analysis of MON 88017: wt CAB Leader + Ract1 Intron Probe

The blot was probed with ^{32}P-labeled wheat CAB leader + rice actin intron (Probe 10, Figure V-1b). Each lane contains ~10 μg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Xho* I)
2: MON 88017 (*Xho* I)
3: MON 88017 (*Pst* I)
4: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
5: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
6: MON 88017 (*Pst* I)
7: MON 88017 (*Xho* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

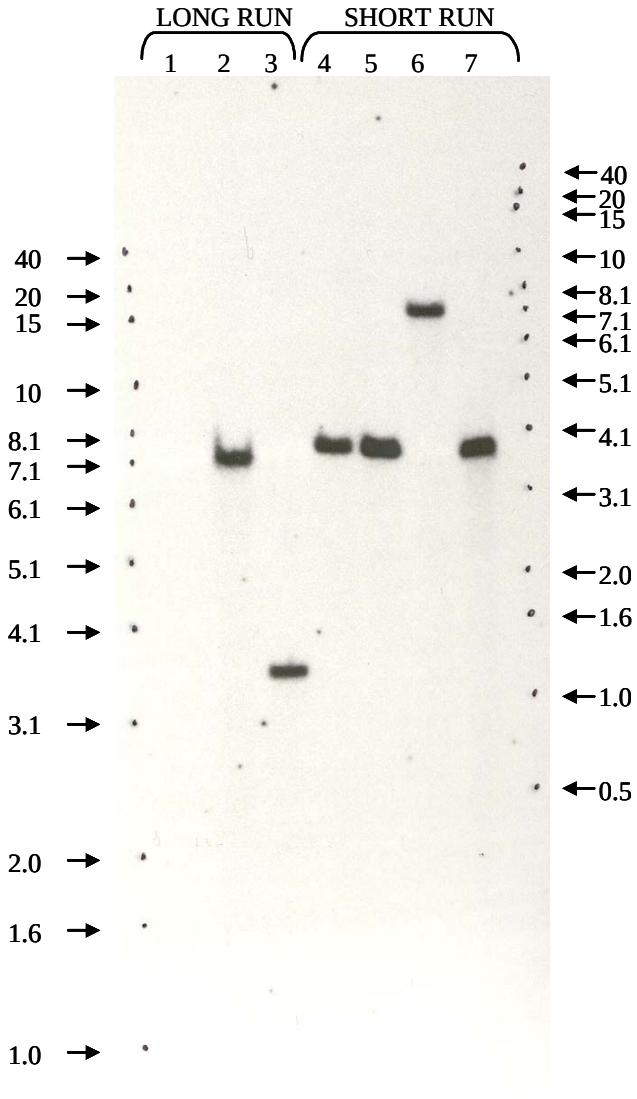


Figure V-9. Southern Blot Analysis of MON 88017: *cry3Bb1* Probe

The blot was probed with ^{32}P-labeled *cry3Bb1* coding region (Probe 11, Figure V-1b). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Xho* I)
2: MON 88017 (*Xho* I)
3: MON 88017 (*Pst* I)
4: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
5: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
6: MON 88017 (*Xho* I)
7: MON 88017 (*Pst* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

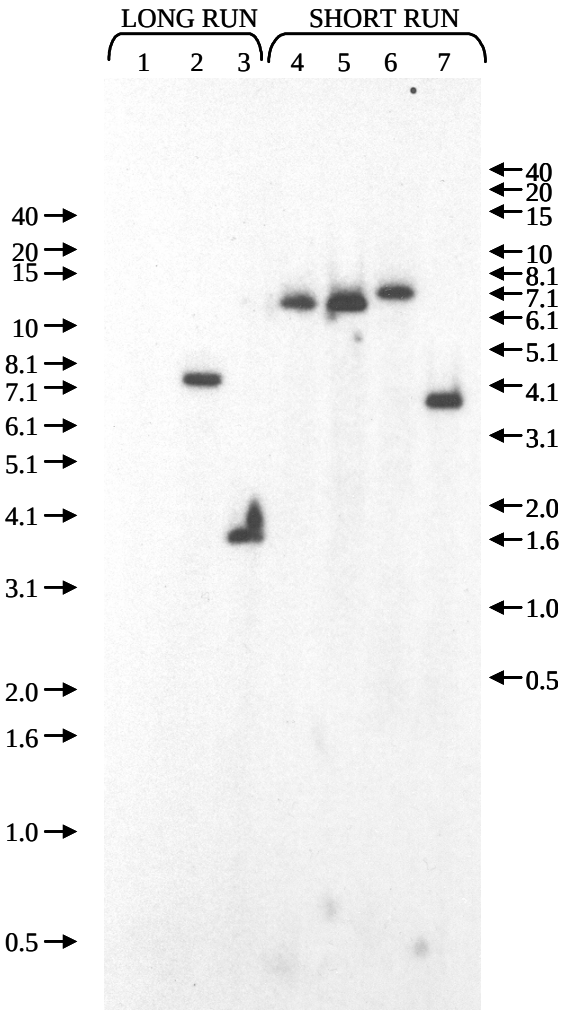


Figure V-10. Southern Blot Analysis of MON 88017: tahsp17 3' Probe

The blot was probed with ^{32}P-labeled tahsp17 3' polyadenylation sequence (Probe 12, Figure V-1b). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control (*Xho* I)
2: MON 88017 (*Xho* I)
3: MON 88017 (*Pst* I)
4: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
5: Control (*Xho* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
6: MON 88017 (*Xho* I)
7: MON 88017 (*Pst* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

V.D. Analysis for Backbone Fragments

For this analysis, MON 88017 and control genomic DNA samples were digested with *Xba* I, which cuts only once within PV-ZMIR39. The blot was hybridized simultaneously with two radiolabeled probes that span the entire backbone sequence in PV-ZMIR39 (Probes 5 and 6, Figure V-1a). The MON 88017 samples were obtained from the same generation used to test for stability of the insert during breeding (Figure V-13). These generations are shown on the MON 88017 breeding tree (Figure V-12). Because of expected cross-hybridization of the probes to the molecular weight marker bands, these lanes were trimmed from the blot prior to hybridization. Aligning these lanes to the blot after hybridization allowed for appropriate annotation of the molecular weight marker band sizes on the film.

The results of this analysis are shown in Figure V-11. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I and mixed with digested control corn genomic DNA (lanes 3 and 4) produced one expected size band at approximately 6.3 kb. The control LH198 DNA (lane 1) produced a diffuse hybridization signal below 1.6 kb, which is likely the result of bacterial contamination of this source of grain. As expected, the second source of control DNA (lane 2) did not produce any detectable hybridization. Genomic DNA from plants representing seven generations of MON 88017 (lanes 5 through 11), including the generation used for molecular characterization (lane 5), also showed no detectable hybridization bands. These results establish that the MON 88017 does not contain any detectable backbone sequences from the transformation vector PV-ZMIR39.

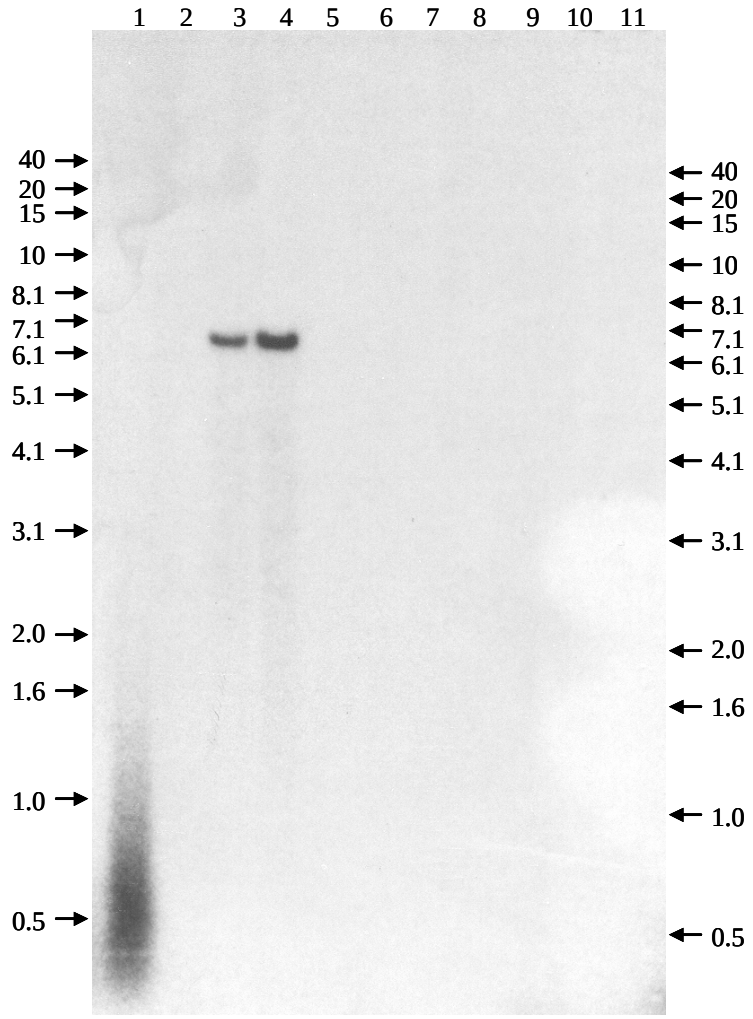


Figure V-11. Southern Blot Analysis of MON 88017: Backbone Analysis

The blot was probed simultaneously with two ^{32}P-labeled probes that span the backbone in PV-ZMIR39 (Probes 5 and 6, Figure V-1a). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

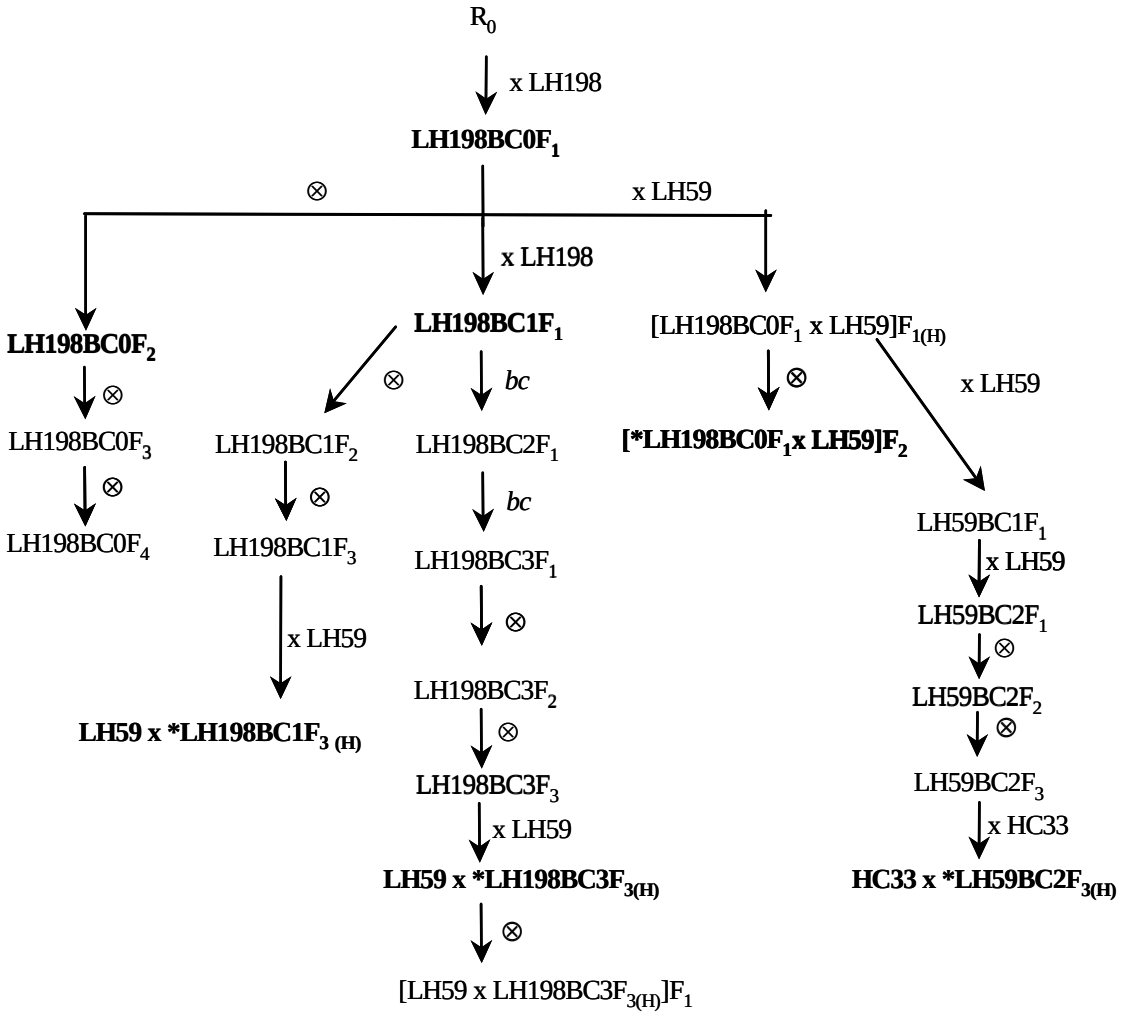
Lane 1: Control LH198 (*Xba* I)
2: Control LH59 x LH198 (*Xba* I)
3: Control LH59 x LH198 (*Xba* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
4: Control LH59 x LH198 (*Xba* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
5: MON 88017 [LH198BC0F_1 x LH59]F_2 (*Xba* I)
6: MON 88017 LH198BC0F_1 (*Xba* I)
7: MON 88017 LH198BC0F_2 (*Xba* I)
8: MON 88017 LH198BC1F_1 (*Xba* I)
9: MON 88017 LH198BC1F_3 x $LH59_{(H)}$ (*Xba* I)
10: MON 88017 LH59 x LH198BC3$F_{3(H)}$ (*Xba* I)
11: MON 88017 HC33 x LH59BC2$F_{3(H)}$ (*Xba* I)

→Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

V.E. Insert Stability in Multiple Breeding Generations

In order to determine generational stability of the integrated DNA, a Southern blot analysis to determine the number of copies of integrated transgenes was performed on multiple generations from the breeding tree of MON 88017. For reference, the breeding tree of MON 88017 is presented in Figure V-12. The specific generations tested are indicated in bold. The (H198BC0F_1 x LH159)F_2 generation, which was used as a test substance in the molecular characterization analyses, was included as a reference in this analysis. For this analysis, MON 88017 and control genomic DNA samples were digested with *Xba* I, which digests only once within PV-ZMIR39. The blot was probed simultaneously with four radiolabeled probes that span the entire length of the insert (Probes 1, 2, 3 and 4, Figure V-1a).

The results of the Southern blot analysis are shown in Figure V-13. Plasmid PV-ZMIR39 DNA previously digested with *Eco*R I, mixed with digested control genomic DNA, produced the expected size bands of approximately 6.3 kb, 3.5 kb and 2.6 kb, correlating to the sizes of PV-ZMIR39 *Eco*R I fragments (lanes 3 and 4). The ~6.3 kb band produced a weaker signal because a smaller portion of the target insert sequence is present on this ~6.3 kb *Eco*R I restriction fragment in comparison to the ~3.5 kb and ~2.6 kb fragments. The previously characterized generation (lane 5) produced the expected size bands of approximately 7.4 kb and 5.5 kb that had been produced in the previous copy number analysis (Figure V-3). In addition, six generations of MON 88017 (lanes 6 through 11) produced the same size bands as the previously characterized generation (~7.4 kb and ~5.5 kb). These results demonstrate that the expected Southern fingerprint of the MON 88017 insert has been maintained across the branches of the breeding tree that were tested. Therefore, the stability of the integrated DNA in MON 88017 has been established over multiple generations.



R_0 - originally transformed plant; ⊗ - self-pollinated; (H) - hybrid; *bc* = backcross;
*MON 88017 side of the hybrid cross

Figure V-12. Breeding Tree of MON 88017

Data	**MON 88017 Generation**
Molecular analysis:	[LH198BC0F1 x LH59]F_2
Insert stability (Southern blot):	Generations bolded
Phenotypic trials (2001):	LH59 x LH198BC1$F_{3\ (H)}$
2002 T/C/R trials:	LH59 x LH198BC3$F_{3\ (H)}$ seed; [LH59 x LH198BC3$F_{3(H)}$]F_1 grain
Expression/composition:	LH59 x LH198BC3$F_{3\ (H)}$ seed; [LH59 x LH198BC3$F_{3(H)}$]F_1 grain
Phenotypic trials (2002):	LH59 x LH198BC3$F_{3\ (H)}$
Dormancy/germination:	LH59 x LH198BC3$F_{3\ (H)}$

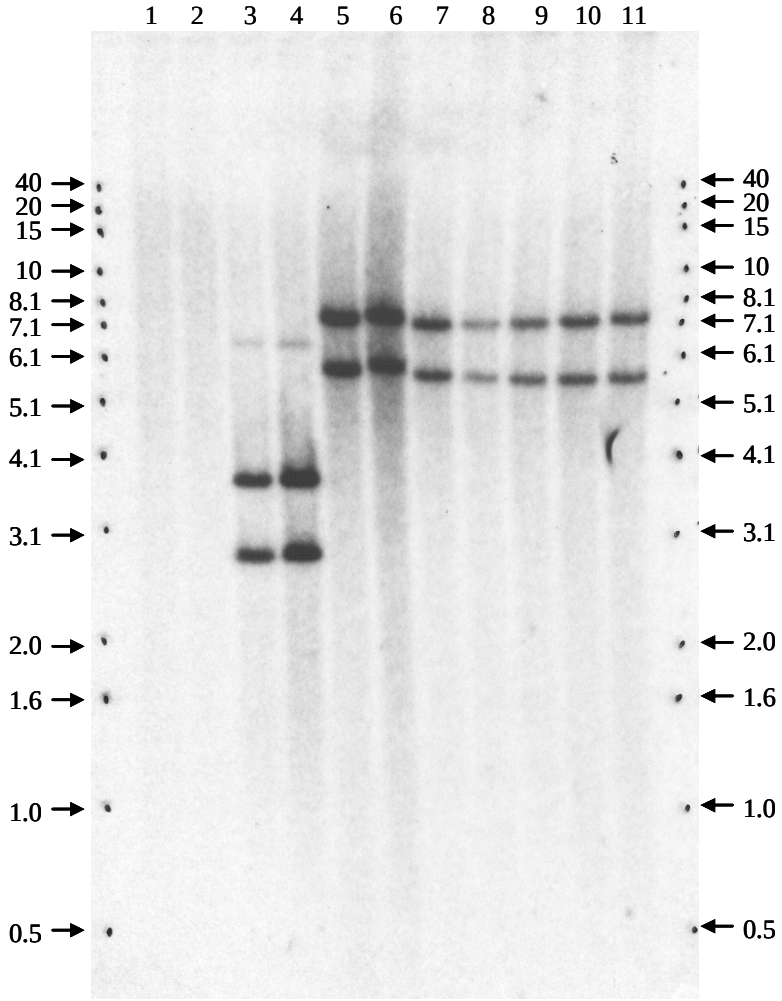


Figure V-13. Stability of the Integrated DNA in MON 88017

The blot was probed simultaneously with four ^{32}P-labeled probes that span the full length of the inserted DNA (Probes 1, 2, 3, and 4, Figure V-1a). Each lane contains ~10 µg of digested genomic DNA isolated from grain. Lane designations are as follows:

Lane 1: Control LH198 (*Xba* I)
2: Control LH59 x LH198 (*Xba* I)
3: Control LH59 x LH198 (*Xba* I) spiked with PV-ZMIR39 (*Eco*R I) [0.5 copy]
4: Control LH59 x LH198 (*Xba* I) spiked with PV-ZMIR39 (*Eco*R I) [1.0 copy]
5: MON 88017 [LH198BC0F_1 x LH59]F_2 (*Xba* I)
6: MON 88017 LH198BC0F_1 (*Xba* I)
7: MON 88017 LH198BC0F_2 (*Xba* I)
8: MON 88017 LH198BC1F_1 (*Xba* I)
9: MON 88017 LH198BC1F_3 x LH59$_{(H)}$ (*Xba* I)
10: MON 88017 LH59 x LH198BC3$F_{3(H)}$ (*Xba* I)
11: MON 88017 HC33 x LH59BC2$F_{3(H)}$ (*Xba* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

V.F. Organization of the Insert in MON 88017

The organization of the elements within the insert in MON 88017 was confirmed using PCR analysis by amplifying seven overlapping regions of DNA that span the entire length of the insert. The location of the PCR products generated in relation to the insert, as well as the results of the PCR analyses, are shown in Figures V-14 and V-15.

The control reactions containing no template DNA (Figure V-14: lanes 4, 7, 10 and 14; Figure V-15: lanes 4, 8 and 11) did not generate PCR products with any of the primer sets, as expected. The control reactions containing control genomic DNA (Figure V-14: lanes 3, 6, 9 and 13) did not generate PCR products with four out of the seven primer sets. The primer set used to generate Products E, F and G did amplify PCR products from the control genomic DNA (Figure V-15: lanes 3, 7 and 10); however, none of these products were of the expected size for the primer set. The same PCR products were also generated using MON 88017 genomic DNA as a template (Figure V-15: lanes 2, 6 and 9). The generation of these PCR products is likely the result of nonspecific amplification of sequences in the corn genome because of one or both of the PCR primers in the primer set.

The plasmid PV-ZMIR39 was used as a positive control in the three PCR analyses (Products C-E) that amplified products containing only inserted DNA rather than the genomic DNA flanking the insert. In these three analyses, amplification from genomic DNA from MON 88017, as well as the plasmid PV-ZMIR39, generated the expected size PCR products of 1.0 kb for Product C (Figure V-14: lanes 8 and 11), 2.0 kb for Product D (Figure V-14: lanes 12 and 15) and 2.3 kb for Product E (Figure V-15: lanes 2 and 5). In the amplification of Product E using plasmid PV-ZMIR39 as template DNA, faint, lower molecular weight products were visible that were most likely because of nonspecific amplification (Figure V-15: lane 5). Amplification from MON 88017 genomic DNA also generated the expected size PCR products of 1.0 kb for Product A (Figure V-14: lane 2); 0.9 kb for Product B (Figure V-14: lane 5); 2.3 kb for Product F (Figure V-15: lane 6); and 2.2 kb for Product G (Figure V-15: lane 9). The generation of the predicted size PCR products from MON 88017 establishes that the arrangement or linkage of elements in the insert is the same as those in plasmid PV-ZMIR39 and that the elements within each gene cassette are arranged as depicted in the schematic of the insert in Figure V-2.

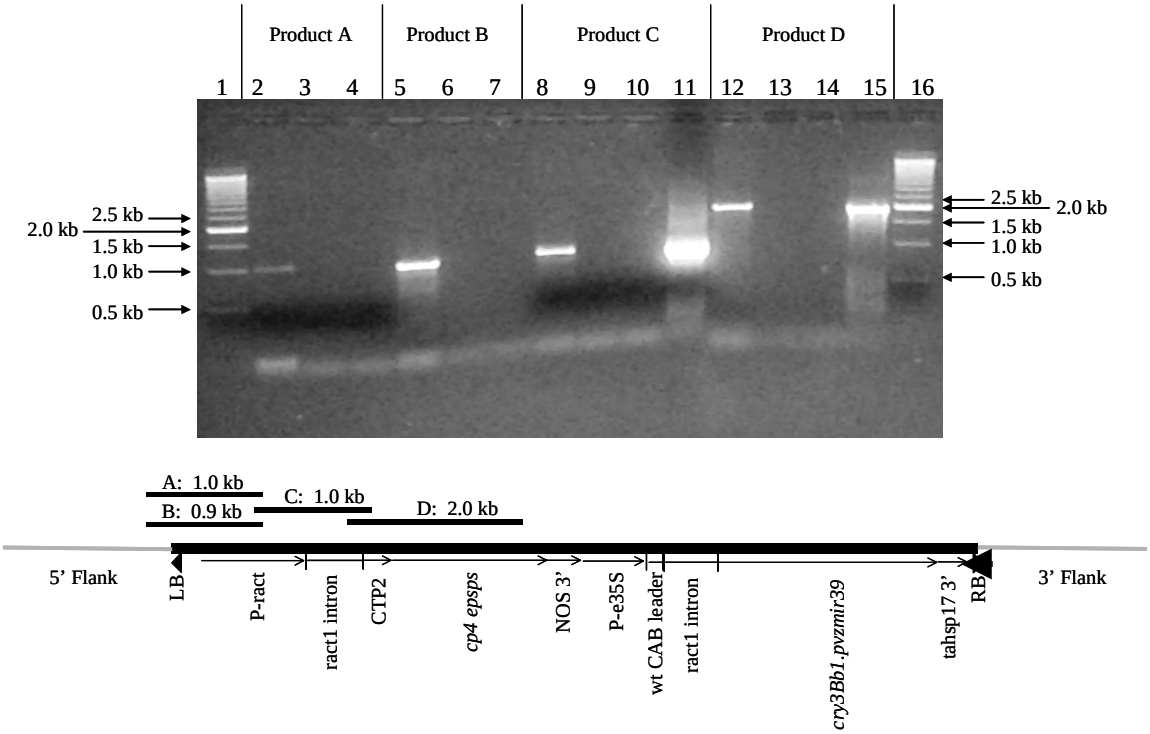


Figure V-14. Overlapping PCR Analyses Across the Insert in MON 88017 (Part I)

PCR analyses demonstrating the linkage of the individual genetic elements within the insert in MON 88017 were performed on plasmid PV-ZMIR39 DNA and on genomic DNA isolated from the grain of MON 88017 and control corn. The expected product size for each amplicon is highlighted in the illustration of the insert in MON 88017 that appears at the bottom of the figure. 20 µl of each of the PCR reactions were loaded on the gel.

Lane designation is as follows:

1: 500 bp DNA ladder (Life Technologies)
2: MON 88017 genomic DNA
3: control LH59 x LH198 DNA
4: control no DNA template
5: MON 88017 genomic DNA
6: control LH59 x LH198 DNA
7: control no DNA template
8: MON 88017 genomic DNA
9: control LH59 x LH198 genomic DNA
10: control no DNA template
11: plasmid PV-ZMIR39 DNA
12: MON 88017 genomic DNA
13: control LH59 x LH198 genomic DNA
14: control no DNA template
15: plasmid PV-ZMIR39 DNA
16: 500 bp DNA ladder (Life Technologies)

⟶ Symbol denotes sizes obtained from MW markers on ethidium bromide stained gel.

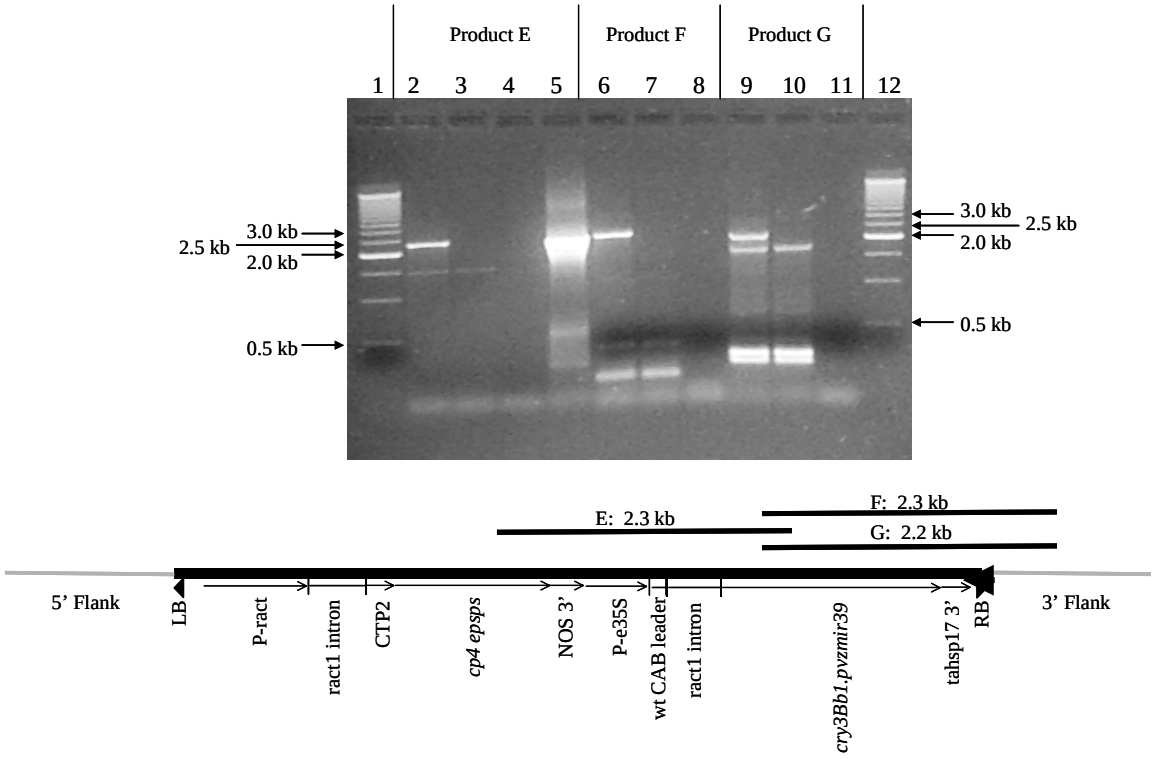


Figure V-15. Overlapping PCR Analyses Across the Insert in MON 88017 (Part II)

PCR analyses demonstrating the linkage of the individual genetic elements within the insert in MON 88017 were performed on plasmid PV-ZMIR39 DNA, conventional corn and MON 88017 genomic DNA extracted from grain. The expected product size for each amplicon is highlighted in the illustration of the insert in MON 88017 that appears at the bottom of the figure. 20 µl of each of the PCR products were loaded on the gel.

Lanes designation is as follows:

1: 500 bp DNA molecular weight ladder (Life Technologies)
2: MON 88017 genomic DNA
3: control LH59 x LH198 genomic DNA
4: control no DNA template
5: plasmid PV-ZMIR39 DNA
6: MON 88017 genomic DNA
7: control LH59 x LH198 genomic DNA
8: control no DNA template
9: MON 88017 genomic DNA
10: control LH59 x LH198 genomic DNA
11: control no DNA template
12: 500 bp DNA molecular weight ladder (Life Technologies)

→ Symbol denotes sizes obtained from MW markers on ethidium bromide stained gel.

V.G. Inheritance of the Corn Rootworm Protection and Glyphosate-Tolerant Traits in MON 88017

Chi square analysis of Mendelian inheritance data over ten generations was performed to determine the heritability and stability of the *cry3Bb1* and *cp4 epsps* genes in the progeny of MON 88017. Expected and observed segregation frequencies of MON 88017 progeny that were positive for the corn rootworm (CRW)-protected and glyphosate-tolerant phenotypes are presented in Table V-1. Plants were identified as positive for the CRW-protected phenotype based on the detection of the Cry3Bb1 protein by ELISA. Because the *cp4 epsps* and *cry3Bb1* genes are linked in the vector used for transformation, and are expected to have identical segregation ratios in the progeny of MON 88017, the results are applicable for inheritance of the glyphosate-tolerant trait in MON 88017.

The breeding history of the ten generations evaluated for Mendelian inheritance is described in Figure V-12.

Genotype frequency was compared by means of a Chi square test (Little and Hills, 1978). The Chi square value (χ^2) was computed as follows:

$$\chi^2 = \Sigma \left[\left(|o - e| - 0.5 \right)^2 / e \right]$$

where, o = observed frequency of the genotype; e = expected frequency of the genotype; and 0.5 = Yates correction factor for analysis with one degree of freedom (df).

Results from the Mendelian heritance analysis are summarized in Table V-1. With only two exceptions, all χ^2 values were less than the critical value of 3.84, thus indicating no significant differences between observed and expected frequency for the CRW-protected/ glyphosate-tolerant phenotypes in eight breeding generations of MON 88017. The results obtained for the LH198BC1F_1 generation were significant at $p \leq 0.05$ (Chi square = 3.84, 1 df) but not at $p \leq 0.01$ (Chi square = 6.63). The results obtained for the LH198BC0F_1 x LH59 generation are attributed to gamete selection caused by Roundup agricultural herbicide application to plants of the previous (LH198BC0F_1) generation. This was conducted to obtain nonsegregating seed for purposes of field evaluation. Gamete selection caused by Roundup agricultural herbicide application has been previously observed for Roundup Ready corn NK603 (USDA Petition 00-011-01p).

Table V-1. Comparison of Expected and Observed Segregation Frequencies for Progeny of MON 88017 Plants

	Observed[a]		Expected[a]		
Generation	+	-	+	-	χ^2
LH198BC$_0$F$_1$	21	14	17.5	17.5	1.03[†]
LH198BC$_0$F$_2$	53	12	48.75	16.25	1.15[†]
LH198BC$_1$F$_1$	21	9	15	15	4.03[#]
LH198BC$_2$F$_1$	10	15	12.5	12.5	0.64[†]
LH198BC$_3$F$_1$	8	5	6.5	6.5	0.31[†]
LH198BC3F$_2$	21	3	18	6	1.39[†]
LH198BC$_0$F$_1$xLH59	29	0	14.5	14.5	27.03[*]
LH59 BC$_1$F$_1$	7	5	6	6	0.08[†]
LH59 BC$_2$F$_1$	8	5	6.5	6.5	0.31[†]
LH59 BC$_2$F$_2$	35	13	36	12	0.03[†]

[a] Symbol (+) denotes number of plants that are positive for the two traits; (-) denotes number of plants that are negative for the two traits.

[†] Not significant at $p \leq 0.05$ (Chi square = 3.84, 1 df)

[#] Significant at $p \leq 0.05$ (Chi square = 3.84, 1 df); but not significant at p≤0.01 (Chi square = 6.63)

[*] Significant at p≤0.05 (Chi square = 3.84, 1 df) and significant at p≤0.01 (Chi square = 6.63).

The results of this analysis are consistent with the finding of a single locus of insertion of the *cry3Bb1* and *cp4 epsps* genes that segregate according to Mendel's laws of genetics. The stability of the insert has been demonstrated through seven generations of cross-fertilization and three generations of self-pollination.

Results of the Southern blot analysis performed on multiple generations from the breeding tree of MON 88017 to determine insert stability were discussed in Section V.E and presented in Figure V-13.

V.H. Conclusions for the Genetic Characterization of MON 88017

MON 88017 was produced by *Agrobacterium*-mediated transformation of corn with the plasmid vector PV-ZMIR39, which contains both the *cp4 epsps* and *cry3Bb1* gene expression cassettes. MON 88017 contains one copy of the introduced DNA at a single integration locus on an approximately 13 kb *Sca* I restriction fragment. No additional elements from the transformation vector PV-ZMIR39, linked or unlinked to intact cassettes, were detected in the genome of MON 88017. PCR analyses were performed to determine the 5' and 3' insert-to-plant junctions and confirm the organization of the elements within the insert. Insert stability analysis demonstrated that the expected Southern blot fingerprint of MON 88017 insert was maintained across the branches of the breeding tree that were tested, thereby confirming the stability of the integrated DNA

over multiple generations. The stability of the insert was also demonstrated through seven generations of cross-fertilization and three generations of self-pollination by the analysis of Mendelian inheritance data. No backbone sequences from the transformation vector PV-ZMIR39 were detected in the tested generations. These data support the conclusion that only the two expected full-length proteins, Cry3Bb1 and CP4 EPSPS, are encoded by the insert in MON 88017.

VI. Characterization of the Introduced CP4 EPSPS and Cry3Bb1 Proteins

This section summarizes the evaluation of the CP4 EPSPS and Cry3Bb1 proteins produced in MON 88017 including: a) equivalence of the *in planta*-produced protein to that of a protein reference standard used in protein safety studies, b) the levels observed in corn tissues, and c) a summary of the food and feed safety assessment provided to the FDA.

VI.A. The CP4 EPSPS Protein

VI.A.1. EPSPS Biochemistry and Mode of Action

The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS: EC2.5.1.19) family of enzymes is ubiquitous to plants and microorganisms. EPSPS has been isolated from both sources, and its properties have been extensively studied (Harrison et al., 1996; Haslam, 1993; Klee et al., 1987; Schonbrunn et al., 2001; Steinrüchen and Amrhein, 1984). The shikimate pathway, and hence the EPSPS protein, is absent in mammals, fish, birds, reptiles, and insects (Alibhai and Stallings, 2001). The bacterial and plant enzymes are monofunctional with molecular mass of 44-48 kDa (Kishore and Shah, 1988). EPSPS proteins catalyze the transfer of the enolpyruvyl group from phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (EPSP) (Alibhai and Stallings, 2001). Because of the stringent specificity for substrates (i.e., EPSPS enzymes bind PEP and S3P), the only known metabolic product produced is EPSP, the penultimate product of the shikimic acid pathway. Shikimic acid is a substrate for the biosynthesis of the aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules. It has been estimated that aromatic molecules, all of which are derived from shikimic acid, represent 35% or more of the dry weight of a plant (Franz et al., 1997).

MON 88017 contains the *cp4 epsps* coding sequence derived from *Agrobacterium sp*. strain CP4. The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). The CP4 EPSPS protein is structurally similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Padgette et al., 1996). In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of EPSP, thereby depriving plants of essential amino acids (Steinrucken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, which are tolerant to Roundup agricultural herbicides, aromatic amino acids and other metabolites that are necessary for growth and development are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996).

VI.A.2. Characterization of the CP4 EPSPS Protein Produced in MON 88017

The CP4 EPSPS protein was purified from the grain of MON 88017 and compared to the CP4 EPSPS isolated from *E. coli* culture. A panel of analytical tests was used to identify,

characterize and compare the plant- and *E. coli*-produced CP4 EPSPS proteins: (1) Western blot analysis and densitometry, (2) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry, (3) matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS), (4) N-terminal sequence analysis, (5) glycosylation analysis, and (6) CP4 EPSPS activity assay. The materials, methods and the results are described in Appendix B. The following summarizes the results and conclusions of this characterization.

In the western blot analysis, conducted with an antibody specific to the CP4 EPSPS protein, a band migrating at approximately 45 kDa was observed in the plant- and *E.coli*-produced protein samples, thereby confirming the identity of the CP4 EPSPS protein produced in MON 88017. The approximate molecular weight of the protein band identified as the plant-produced CP4 EPSPS protein, estimated by comparison to molecular weight markers on a Colloidal Brilliant Blue G stained SDS-polyacrylamide gel, was 45 kDa. The purity of the plant-produced CP4 EPSPS protein was 45.5%, as estimated by densitometry. The molecular weight of the plant-produced CP4 EPSPS protein, as determined using the mass average (MH+) molecular weight from MALDI-TOF MS, was 47,447.25 Da. MALDI-TOF mass spectrometry analysis of the plant-produced CP4 EPSPS protein after trypsin digestion yielded peptide masses encompassing 55.2% of the deduced amino acid sequence (251/455 amino acids) (Figure IV-1). The N-terminal sequence of this 45 kDa protein band was consistent with the N-terminal sequence of the CP4 EPSPS protein. The functional activities of the plant- and *E. coli*-produced CP4 EPSPS proteins were determined using a phosphate release assay and shown to be similar. The specific activities of the plant- and *E. coli*-produced CP4 EPSPS proteins were 7.1 U/mg and 6.0 U/mg, respectively.

These results lead to the conclusion that the plant-produced CP4 EPSPS protein is equivalent to the *E. coli*-produced CP4 EPSPS protein.

VI.A.3. Safety Assessment Summary of the CP4 EPSPS Protein

The EPA previously has reviewed and established an exemption from the requirement of a tolerance for CP4 EPSPS and the genetic material necessary for the production of this protein in or on all raw agricultural commodities (40 CFR §180.1174). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, the demonstration of no homology to known toxins and allergens, and lack of toxicity in a acute oral mouse gavage study.

In the comprehensive food and feed safety and nutritional assessment of MON 88017 submitted to the FDA on March 31, 2004, the following conclusions were reached regarding the safety assessment of the CP4 EPSPS protein:

a) The donor organism, *Agrobacterium* sp. strain CP4, is not known for human or animal pathogenicity and is not commonly allergenic. Additionally, *Agrobacterium* sp. strain CP4, and the CP4 EPSPS protein it produces, have been reviewed previously as a part of the safety assessment for other Roundup Ready crops.

b) A history of the safe use of CP4 EPSPS protein has been demonstrated, based on the similarity of the CP4 EPSPS protein in MON 88017 to EPSPS naturally present in food crops (e.g., soybean and corn) and in microbial food sources such as bakers yeast (*Saccharomyces cerevisiae*), and to the CP4 EPSPS protein produced in a number of other Roundup Ready crops, including soybean, NK603 corn, cotton and canola. The CP4 EPSPS protein is functionally equivalent to native plant EPSPS except for lack of affinity for glyphosate.
c) The CP4 EPSPS protein purified from *E. coli* was found to be physicochemically and functionally equivalent to the protein produced in MON 88017.
d) Large margins of exposure for CP4 EPSPS protein indicate that there is no significant risk to human and animal health associated with dietary exposure to food and feed products derived from MON 88017.
e) The CP4 EPSPS protein is rapidly degraded in simulated digestive fluids and would be unlikely to elicit potential toxic effects.
f) No biologically relevant structural similarities were observed between the CP4 EPSPS protein and pharmacologically active proteins that are known to cause adverse health effects in humans or animals.
g) Results from the acute oral toxicity study demonstrated that the CP4 EPSPS protein is not acutely toxic and does not cause any adverse effects.

These conclusions establish the food and feed safety of the CP4 EPSPS protein produced in MON 88017.

VI.B. The Cry3Bb1 Protein

VI.B.1. *B.t.* Cry Proteins and Mode of Action

The *B.t.* Cry proteins comprise at least 44 primary classes of Cry proteins (Cry1 – Cry44) and two primary classes of cytolytic, or Cyt, proteins (Cyt1, Cyt2), ranging in molecular mass size from 25 kDa to over 130 kDa (Crickmore et al., 1998 and 2004). The current nomenclature, based on amino acid identity, allows closely related proteins to be ranked together. Each new protein is assigned a unique name incorporating four hierarchical ranks consisting (in order) of an Arabic numeral, uppercase letter, lowercase letter, and an Arabic numeral (e.g., Cry3Bb1 protein). Thus, proteins with identities of:

a) <45% differ in primary rank (e.g., Cry2, Cry3, etc),
b) >45% but <78% differ in secondary rank (e.g., Cry3A, Cry3B),
c) >78% but <95% differ in tertiary rank (e.g., Cry3Ba, Cry3Bb), and
d) >95% differ in quaternary rank (e.g., Cry3Bb1, Cry3Bb2) and are considered allelic variants.

In general, the *B.t.* protein primary rank denotes specific insecticidal activity; for example, Cry1, Cry2, Cry3, and Cry4 proteins are toxic to lepidopteran, lepidopteran/dipteran, coleopteran, and dipteran pests, respectively (Bravo, 1997; Höfte and Whitely, 1989).

A generalized mode of action for Cry proteins has been described by English and Slatin (1992). It includes ingestion of the crystals by insects, solubilization of the crystals in the insect midgut, and proteolytic processing of the released Cry protein by digestive enzymes, sometimes with partial digestion activating the toxin. The activated protein diffuses through the peritrophic membrane of the insect to the midgut epithelium. There it binds to specific high-affinity receptors on the surface of the midgut epithelium of target insects (Hoffman et al., 1988a and 1988b; Van Rie et al., 1989 and 1990; Wolfersberger et al., 1986). Pores are formed in the membrane, leading to leakage of intracellular contents (e.g., K^+) into the gut lumen and water into the epithelial gut cells (Sacchi et al., 1986). The larval gut epithelial cells swell because of osmotic pressure and lyse. The gut becomes paralyzed as a consequence of changes in electrolytes and pH, causing the larval insect to stop eating and die.

Receptor binding is a critical step in the mechanism of action for Cry proteins. Irreversible binding of these proteins to midgut receptors appears to be correlated with insect susceptibility to the toxin (Schnepf et al., 1998). This observation is relevant to assessing the safety of Cry proteins for humans because no receptors for these proteins have been identified on intestinal cells of mammals (Noteborn, 1994; Sacchi et al., 1986; Van Mellaert et al., 1988). This would explain, at least in part, the absence of any reported adverse effects for *B.t.* products in humans.

VI.B.2. The Cry3 Class of Proteins

The Cry3 class of proteins share >45% amino acid sequence homology and have coleopteran-specific activity. Two Cry3 class proteins, Cry3Aa4 and Cry3Bb1, have been registered and exempted from the requirement of a tolerance by the EPA. Cry3Aa4 has been commercialized in the U.S. and other countries as a microbial-derived *B.t.* mixture, Foil® Biological Insecticide, and more recently, when expressed in genetically enhanced potatoes to control the coleopteran pest, Colorado potato beetle (CPB) (Perlak et al., 1993*)*. Cry3Bb1, one of the active ingredients in the microbial pesticide mixture Raven Oil Flowable Bioinsecticide was commercialized in the U.S. in 1995 to control CPB in various crops (Baum et al., 1996). In 2003, Monsanto commercialized YieldGard Rootworm corn (MON 863) in the U.S. that expresses a Cry3Bb1 protein for the control of corn rootworm species, based on the discovery in 1991 of a *B.t.* strain containing an insecticidal Cry3Bb1 protein.

The Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of 99.8%, differing from one another by only one of 653 amino acids. The single difference occurs at position 166; in MON 88017, there is an aspartic acid at this position that is also present in the wild-type Cry3Bb1 protein, while in MON 863 the aspartic acid is substituted by a glycine (see Figure IV-2). The subsequent sections describe the physicochemical characterization and functional activity of the Cry3Bb1 protein produced in MON 88017, and the basis for its equivalency to the Cry3Bb1 protein produced in MON 863.

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VI.B.3. Characterization of the Cry3Bb1 Protein Produced in MON 88017

A series of analyses were conducted to characterize the Cry3Bb1 protein isolated from the grain of MON 88017 and to establish its equivalence to the *E. coli*-produced Cry3Bb1 protein standard that was used in studies to establish its safety. The tests included: (1) Western blot analysis and densitometry, (2) SDS-PAGE and densitometry, (3) MALDI-TOF mass spectrometry, (4) glycosylation analysis, and (5) a Cry3Bb1 bioactivity assay. The materials, methods, and key results are described in Appendix C.

Protein bands migrating at approximate molecular weights of 77, 66, and 55 kDa were identified as plant-produced Cry3Bb1 protein fragments using western blot analysis with anti-Cry3Bb1 antibody. The purity, calculated as the sum of the percent optical density of the three bands identified as Cry3Bb1 protein, was 66.1%. The approximate molecular weights of each of the three Cry3Bb1 protein bands identified in the plant-produced Cry3Bb1 protein sample, estimated by comparison to molecular weight markers on a Colloidal Brilliant Blue G stained SDS-polyacrylamide gel, were 77, 66, and 55 kDa. MALDI-TOF mass spectrometry analysis of these bands after trypsin digestion yielded peptide masses consistent with the peptide masses expected for the Cry3Bb1 protein. Together, masses identified for each protein band yielded 44% overall coverage (287 out of 653 amino acids) of the peptide sequence of the plant-produced Cry3Bb1 protein. The plant-produced Cry3Bb1 protein was not glycosylated. The functional activities of the plant- and *E. coli* - produced Cry3Bb1 proteins determined by insect bioassay were not significantly different ($p>0.05$), leading to the conclusion that the proteins are functionally equivalent.

Collectively, these data establish the physicochemical and functional properties of the Cry3Bb1 protein produced in MON 88017 based on its equivalence to the *E. coli*-produced Cry3Bb1 protein used in toxicology and other studies to establish its safety.

VI.B.4. Safety Assessment Summary of the Cry3Bb1 Protein

The EPA has established an exemption from the requirement of a tolerance for residues of the Cry3Bb1 protein and the genetic material for its production in corn, (40 CFR §180.1174; EPA, 2004). This exemption was based on a safety assessment, which concluded that the Cry3Bb1 is not toxic to mammals, is rapidly and completely digested in simulated gastric fluid, has no homology to known toxins and allergens, and is unlikely to produce a toxic or allergenic response in humans.

In the comprehensive food and feed safety and nutritional assessment of MON 88017 submitted to the FDA on March 31, 2004, the following conclusions were reached regarding the safety assessment of the Cry3Bb1 protein:

a) The donor organism, *Bacillus thuringiensis*, has been used commercially in the U.S. since 1958 to produce microbial-derived products with insecticidal activity. The extremely low mammalian toxicity of *B.t.*-based insecticide products has been demonstrated in numerous safety studies, and there are no confirmed cases of allergic reactions to Cry proteins in applicators of microbial-derived *B.t.* products during 40 years of use.

a) A history of safe use of Cry3Bb1 protein has been demonstrated, based on similarity to the Cry3Bb1 protein produced in the YieldGard Rootworm corn (MON 863) and to the wild type Cry3Bb1 protein present in Raven bioinsecticide.
b) The Cry3Bb1 protein purified from *E. coli* was found to be physicochemically and functionally equivalent to the protein produced in MON 88017.
c) Large margins of exposure for the Cry3Bb1 protein indicate that there is no significant risk to human and animal health associated with dietary exposure to food and feed products derived from MON 88017.
d) The Cry3Bb1 protein is rapidly degraded in simulated digestive fluids and would be unlikely to elicit potential toxic effects. This is consistent with the results of the studies conducted for Cry3Bb1 protein produced in YieldGard Rootworm corn (MON 863).
e) No biologically relevant structural similarities were observed between the Cry3Bb1 protein and pharmacologically active proteins that are known to cause adverse health effects in humans or animals.
f) Results from the acute oral toxicity study demonstrate that the Cry3Bb1 protein is not acutely toxic and does not cause any adverse effects.

These conclusions establish the food and feed safety of the Cry3Bb1 protein produced in MON 88017.

VI.C. Levels of the CP4 EPSPS and Cry3Bb1 Proteins Produced in MON 88017

The levels of the CP4 EPSPS and Cry3Bb1 proteins in various tissues of MON 88017 were estimated using enzyme-linked immunosorbent assay (ELISA). The materials and methods for the ELISA analysis, as well as a description of the tissue types, are provided in Appendix D. To produce the tissues for analysis, MON 88017 and conventional corn were planted at three field locations during the 2002 growing season. The sites were located in the major corn-growing region of the United States. A randomized complete block design with three replications was used at all sites. Pollen, silk, forage, forage root, grain, stover and senescent root were collected at appropriate times of plant development. CP4 EPSPS and Cry3Bb1 protein levels in these tissues are presented in Table VI-1. The following tissues were collected over the season from the V2 to the R1 vegetative growth stages: overseason leaf samples (OSL 1-4), overseason whole plant (OSWP 1-4), and overseason root (OSR 1-4). The levels of CP4 EPSPS and Cry3Bb1 proteins in overseason tissues are presented in Tables VI-2 and VI-3, respectively. Limits of detection and quantitation are presented in Table VI-4.

The mean CP4 EPSPS protein levels across all sites were 220 µg/g dwt in young leaf, 390 µg/g dwt in pollen, 57 µg/g dwt in forage, 70 µg/g dwt in forage root, and 5.8 µg/g dwt in grain. CP4 EPSPS levels were not assessed in whole plant, silk and stover. In tissues harvested throughout the growing season, mean CP4 EPSPS protein levels across all sites ranged from 150-220 µg/g dwt in leaf and 70-150 µg/g dwt in root. In general, levels of the CP4 EPSPS protein declined over the growing season.

The mean Cry3Bb1 levels across all sites were 570 µg/g dwt in young leaf, 25 µg/g dwt in pollen, 380 µg/g dwt in silk, 95 µg/g dwt in forage, 130 µg/g dwt in forage root, 15 µg/g dwt in grain, and 88 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry3Bb1 protein levels across all sites ranged from 260-570 µg/g dwt in leaf, 220-500 µg/g dwt in the whole plant, and 100-370 µg/g dwt in root tissues. In general, levels of the Cry3Bb1 protein declined over the growing season.

Table VI-1. Levels of the CP4 EPSPS and Cry3Bb1 Proteins in Tissues of MON 88017

Tissue Type[1]	Growth Stage	Cry3Bb1 Mean (SD)[2] [range][3]		CP4 EPSPS Mean (SD)[2] [range][3]	
		(µg/g dwt)	(µg/g fwt)	(µg/g dwt)	(µg/g fwt)
Young leaf	V2-V3 (14-22 DAP)	570 (170) [230-820]	76 (23) [28-110]	220 (30) [160-260]	30 (5.3) [19-36]
Pollen	R1 (62-69 DAP)	25 (4.2) [17-32]	14 (2.5) [11-20]	390 (85) [210–470]	220 (43) [130-280]
Silk	R1 (62-69 DAP)	380 (65) [300-500]	37 (5.6) [30-45]	NM	NM
Forage	R4-R6 (early dent) (97-124 DAP)	95 (19) [75-130]	27 (5.5) [22-39]	57 (7.6) [42-69]	16 (2.1) [12-19]
Forage root	R4-R6 (early dent) (97-124 DAP)	130 (29) [98-170]	21 (3.1) [17-27]	70 (20) [47-110]	11 (2.8) [6.6-15]
Grain	R6 (133–146 DAP)	15 (3.6) [10-22]	13(3.1) [8.7-19]	5.8 (0.97) [4.1-7.1]	5.1 (0.89) [3.7-6.3]
Stover	R6 (after harvest) (133-147 DAP)	88 (13) [71-110]	30 (4.4) [25-39]	NM	NM

[1] A description of the tissue types is provided in Appendix D. Limits of detection and quantitation are described in Table VI-4.
[2] The mean and standard deviation were calculated across sites and replicates (n=9).
[3] Minimun and maximum values were determined for each tissue type across sites.

DAP = days after planting
n = number of samples
SD = standard deviation. The mean and SD were calculated across sites (n=9).
NM = not measured

Table VI-2. Levels of the CP4 EPSPS Protein in Overseason Tissues of MON 88017[1,2]

Tissues[3] **(n=9)**		**V2-V3 (14-22 DAP)**	**V5 (26-34 DAP)**	**V8 (40-45 DAP)**	**V11-V17 (55-62 DAP)**	**R4-R6 (97-124 DAP)**
µg/g dwt						
Leaf	Mean (SD)	220 (30)	190 (26)	170 (37)	150 (19)	NA
	Range	160-260	130-250	140-240	120-170	
Root	Mean (SD)	150 (34)	110 (29)	100 (30)	97 (18)	70 (20)
	Range	110-220	74-160	62-160	72-130	47-110
µg/g fwt						
Leaf	Mean (SD)	30 (5.3)	36 (5.5)	38 (8.6)	35 (4.7)	NA
	Range	19-36	29-46	28-54	30-42	
Root	Mean (SD)	16 (3.3)	15 (3.3)	13 (3.0)	14 (2.3)	11 (2.8)
	Range	10-21	12-20	8.9-18	11-18	6.6-15

[1]DAP = days after planting; dwt = dry weight of tissue; fwt = fresh weight of tissue; n = number of samples; NA = not applicable; SD = standard deviation.

[2]A description of the tissue types is provided in Appendix D. Limits of detection and quantitation are described in Table VI-4.

[3]Growth stages V2-V3, V5, V8 and V11-V17 correspond to over-season tissue samples -1, -2, -3 and -4, respectively (e.g., OSL-1, etc.). The root samples collected at the R4-R6 and R6 growth stages correspond to forage root and senescent root, respectively.

Table VI-3. Levels of the Cry3Bb1 Protein in Overseason Tissues of MON 88017[1,2]

Tissues[3] (n=9)		V2-V3 (14-22 DAP)	V5 (26-34 DAP)	V8 (40-45 DAP)	V11-V17 (55-62 DAP)	R4-R6 (97-124 DAP)	R6 (133-147 DAP)
µg/g dwt							
Leaf	Mean (SD)	570 (170)	430 (58)	310 (45)	260 (44)	NA	NA
	Range	230-820	310-510	240-380	190-340		
Whole plant	Mean (SD)	500 (64)	380 (170)	310 (48)	220 (23)	NA	NA
	Range	410-590	150-600	230-380	190-250		
Root	Mean (SD)	370 (80)	250 (71)	210 (78)	180 (37)	130 (29)	100 (19)
	Range	240-510	190-420	150-410	110-230	98-170	77-140
µg/g fwt							
Leaf	Mean (SD)	76 (23)	75 (10)	69 (12)	62 (9.2)	NA	NA
	Range	28 - 110	58 - 92	55 - 90	49 - 77		
Whole plant	Mean (SD)	50 (6.4)	37 (8.0)	34 (5.2)	32 (4.4)	NA	NA
	Range	41 - 59	26 - 48	25 - 42	26 - 38		
Root	Mean (SD)	39 (8.1)	34 (8.4)	29 (8.2)	26 (5.4)	21 (3.1)	18 (2.6)
	Range	24 - 51	25 - 55	21 - 50	16 - 34	17 - 27	14 - 22

[1]DAP = days after planting; dwt = dry weight of tissue; fwt = fresh weight of tissue; n = number of samples; NA = not applicable; SD = standard deviation.

[2]A description of the tissue types is provided in Appendix D. Limits of detection and quantitation are described in Table VI-4.

[3]Growth stages V2-V3, V5, V8 and V11-V17 correspond to over-season tissue samples -1, -2, -3 and -4, respectively (e.g., OSL-1, etc.). The root samples collected at the R4-R6 and R6 growth stages correspond to forage root and senescent root, respectively.

Table VI-4. ELISA Limits of Detection and Quantitation for the CP4 EPSPS and Cry3Bb1 Proteins

Tissue[1]	Cry3Bb1		CP4 EPSPS	
	LOD (µg/g fwt)	LOQ (µg/g fwt)	LOD (µg/g fwt)	LOQ (µg/g fwt)
Leaf	0.0073	0.044	0.090	0.17
Root	0.032	0.040	0.050	0.10
Pollen	0.020	0.039	0.12	0.18
Silk	0.012	0.041	NM	NM
Forage	0.010	0.047	0.11	0.21
Grain	0.0097	0.051	0.18	0.28

[1]A description of the tissue types is provided in Appendix D. Whole plant and stover were analyzed using the forage assay. Senescent root was analyzed using the root assay.

LOD = limit of detection.
LOQ = limit of quantitation.
NM = not measured.

VII. Phenotypic, Ecological and Compositional Assessment

This section provides a phenotypic, ecological and compositional assessment of the regulated article MON 88017 to reach a determination that it is no more likely to pose a plant pest risk than conventional corn.

VII.A. Phenotypic and Ecological Assessment

VII.A.1. Phenotypic and Ecological Characteristics

In the phenotypic and ecological assessment of MON 88017, data were collected that address the specific characteristics suggested by USDA-APHIS. These characteristics have been grouped into five general categories: 1) dormancy and germination, 2) emergence and vegetative growth; 3) reproductive growth; 4) seed retention; and 5) disease, insect, and abiotic stressor-plant interactions. An overview of the characteristics assessed is presented in Table VII-1.

The phenotypic evaluation is based on both laboratory experiments and replicated, multi-site field trials conducted over two years by agronomists and other scientists who are familiar with the production and evaluation of corn. In each of these assessments, the regulated article, MON 88017, was compared to an appropriate conventional control and reference materials to provide benchmark values common to conventional corn for each measured phenotypic and ecological characteristic. Further descriptions of the test, control and reference materials are provided in the methods and materials section for each experiment (Appendices E and F).

Table VII-1. Phenotypic and Ecological Characteristics Measured for MON 88017

General Characteristic	Characteristic Measured	Evaluation timing	Evaluation description
Dormancy / Germination	Dormancy, Germination	After 4 and 7 days	Percent normal germinated, abnormal germinated, viable hard (dormant), dead, and viable firm swollen seed
Emergence	Seedling vigor	Stage V2 – V3	Rated on a 0-9 scale, where 0 = dead and 9 = most vigorous growth[1]
	Early stand count	Stage V2 - V4[2]	Number of emerged plants per plot
Vegetative Growth	Final stand count	Pre-harvest	Number of plants per plot
	Stay green	Maturity	Rated on a 0-9 scale, where 0 = entire plant is dried and 9 = entire plant is green[3]
	Ear height	Maturity	Distance from the soil surface at the base of the plant to the ear attachment node
	Plant height	Maturity	Distance from the soil surface at the base of the plant to the flag leaf collar
	Stalk lodged plants	Pre-harvest	Number of plants per plot broken below the ear
	Root lodged plants	Pre-harvest	Number of plants per plot leaning at the soil surface at >30° from the vertical
Reproductive Growth	Days to 50% pollen shed	Pollen shed	Days from planting until 50% of the plants have begun to shed pollen
	Days to 50% silking	Silking	Days from planting until 50% of the plants have silks exposed
	Pollen viability	Tasseling	Viable and nonviable pollen based on pollen grain staining characteristics
	Pollen morphology	Tasseling	Diameter of viable pollen grains
	Grain moisture	Harvest	Moisture percentage of harvested shelled grain
	Test weight	Harvest	Test weight of harvested shelled grain
	Yield	Harvest	Harvested shelled grain, adjusted to 15.5% moisture[4]
Seed Retention	Dropped ears	Pre-harvest	Number of mature ears dropped from plants
Plant-ecological interactions	Insect, disease and abiotic stressors	Planting to harvest	Qualitative assessment of each plot, with rating on a 0-9 scale for insect, disease, and abiotic stressors

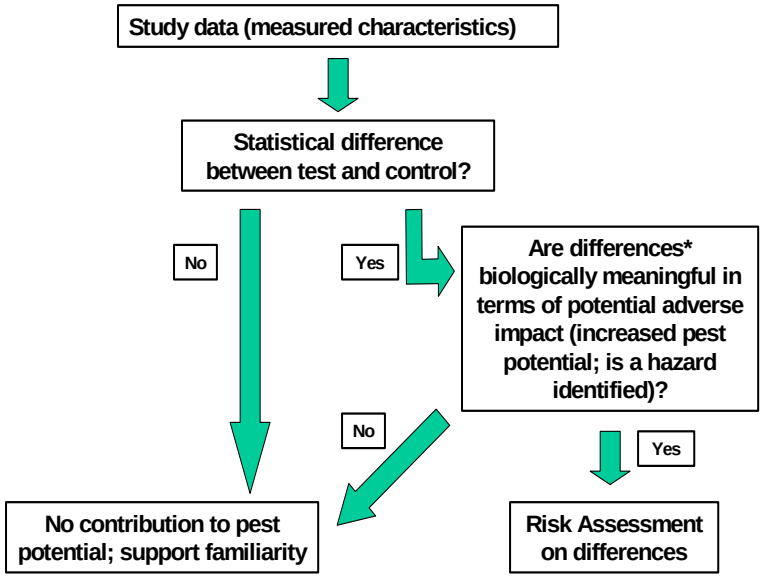
[1]In the 2001 field trials, the ratings were, 1= excellent and 9 = poor growth.
[2]In the 2001 field trials, early stand count was recorded at the V4-V6 growth stage.
[3]In the 2001 field trials, the ratings were, 1= 90-100% green tissue and 9 = 0-19% green tissue.
[4]In the 2001 field trials, yield was measured at a grain moisture of 13%.

VII.A.2. Interpretation of Phenotypic and Ecological Interaction Data

Phenotypic, agronomic, and ecological data are useful to support the concept of equivalence and familiarity as it relates to an ecological risk assessment. On the basis of these data, one can establish if there is an increased pest potential of MON 88017, and if the phenotype has been unintentionally changed beyond the introduced traits.

Measurement of phenotypic characteristics and ecological interactions provides data for a comparative assessment of ecological risk (pest potential) between a biotechnology-derived crop and an appropriate control. A tiered approach is used to assess whether a difference is or is not biologically meaningful. As such, evaluation of phenotypic characteristics is designed according to the biology of the crop using replicated plots at multiple locations with appropriate controls and commercial crop references. If no statistically significant differences are detected between the biotechnology-derived crop and an appropriate control, a conclusion of no difference in pest potential can be made. If a statistically significant and biologically meaningful difference in a characteristic is detected, the magnitude of the difference is considered (relative to the known ranges of values for the crop), and its effect on pest potential is assessed, as shown in the schematic diagram below.

Schematic diagram of data interpretation methods:



*Consider direction & magnitude of change and interaction of differences

A statistically significant change in one characteristic is considered in terms of the direction of the change (i.e., contributing to or detracting from pest potential), its magnitude (outside the range of the control or reference organisms), and within the

context of other observed changes. Interpretation of any detected differences in ecological risk assessment data should focus on those that are biologically meaningful (i.e., contribute to pest potential). Differences detected in a characteristic are considered alone and in the context of 1) whether or not trends are observed over locations; 2) differences that are detected in other measured characteristics; 3) contributions to enhanced pest potential of the crop itself; and 4) potential effects of the transfer of the trait to a wild or weedy species.

For example, a significant difference in a growth characteristic may not be biologically meaningful in terms of weed potential if it is not outside the range typical for that crop or if a change in another parameter is in the direction toward lower weed potential. A careful assessment must be used to distinguish between meaningful changes toward increased pest potential and differences associated with natural plant variation or random experimental error. A finding of no meaningful difference can be concluded only after an evaluation of all the data collected on the characteristics measured.

The following sections describe the experiments conducted for the phenotypic and ecological evaluation of MON 88017. The USDA notifications for the field trial locations are listed in Table VII-2. The purpose of these experiments was to assess whether the presence of the *cp4 epsps* and *cry3Bb1* genes, the presence of the gene products (Cry3Bb1 and CP4 EPSPS proteins), or the genetic modification process altered the phenotypic and ecological characteristics of MON 88017 compared to conventional control corn. The sites selected for these evaluations represent a wide range of environments that would be encountered for corn grown in the U.S.

Table VII-2. USDA Notifications and Field Trial Locations for Phenotypic and Ecological Assessment of MON 88017

USDA-APHIS #	Site State	Site County	Location Code
Dormancy/Germination (seed production)			
02-267-03[1]	HI	na	na
02-267-03[1]	PR	na	na
2001 Phenotypic Trials			
01-023-12n	Nebraska	York	YK
01-026-17n	Illinois	Jersey	FL
01-026-17n	Illinois	Warren	JE
01-026-17n	Illinois	Warren	LA
01-026-17n	Illinois	Henderson	MN
01-026-17n	Illinois	McDonough	RA
01-026-17n	Illinois	Stark	SC
01-026-17n	Illinois	Champaign	TL
2002 Phenotypic Trials			
02-042-18n	Missouri	Shelby	BE
02-042-18n	Missouri	Shelby	CL
02-043-04n	Illinois	Clinton	CR
02-042-17n	Illinois	Warren	MN
02-037-04n	Indiana	Hamilton	NB
02-036-03n	Iowa	Jefferson	RL
02-036-03n	Iowa	Benton	VH
02-036-03n	Iowa	Hamilton	WC
02-066-14n	Illinois	Stark	WY
02-036-05n	Nebraska	York	YK
2002 T/C/R Production Trials[2]			
02-022-27n	Iowa	Benton	IA
02-022-52n	Illinois	Stark	IL
02-022-51n	Nebraska	York	NE
02-023-10n	Ohio	Fayette	OH

[1]USDA notification for movement of seed only; na = not applicable.
[2]Test/control/reference tissues harvested for expression, composition and other studies.

VII.A.3. Dormancy and Germination Evaluation

Seed dormancy is an important characteristic that is often associated with plants that are weeds (Anderson, 1996). Dormancy mechanisms, including hard seed, vary with species and tend to include complex processes. For most crops, including corn, the number of hard seed is negligible or nonexistent. Standardized germination assays are routinely used to measure the germination potential of corn seed (AOSA, 1998).

Starting seed materials were produced by Monsanto in 2002 at two production locations, Puerto Rico and Hawaii (Table VII-2). Seed materials included one test (MON 88017),

one control, and three reference materials from each production location. The test and control materials were the positive (LH59 x MON 88017[+]/LH198BC3F_3) test and negative (LH59 x MON 88017[-]/LH198BC3F_3) control isolines, respectively, having the same genetic background (see Figure V-12).

Evaluations were conducted in temperature-controlled growth chambers using rolled towel tests to measure dormancy and germination characteristics. Four replicates of MON 88017, control, and three reference corn seeds were tested in seven growth chambers, each maintained in the dark under one of the following temperature regimes:

- Constant target temperature of approximately 5, 10, 20, or 30°C
- Alternating target temperatures of approximately 5/20, 10/20, or 20/30°C.

The Association of Official Seed Analysis (AOSA) recommends a temperature range between 20-30°C as optimal for germination of corn (AOSA, 1998). Temperatures <20°C were included to detect dormant seeds since most seeds would be expected to germinate at the optimal temperatures. The following five germination characteristics were evaluated: percent normal germinated seed, percent viable hard seed, percent abnormal germinated seed, percent viable firm swollen seed, and percent dead seed. A statistical comparison between the test and control materials was conducted using a Statistical Analysis Software program (SAS Version 8.2, 1999-2001). Statistical significance was set at $p \leq 0.05$. A summary of the results is provided below; details, including data tables, can be found in Appendix E.

Out of 70 comparisons of germination characteristics made between MON 88017 and control corn, 61 were not statistically different at $p \leq 0.05$. No hard seed were observed and no differences in percent viable hard seed were detected between MON 88017 and the control from either location. Statistically significant differences observed between MON 88017 and the control corn were as follows: one each for percent normal germinated seed and percent dead seed, two for percent abnormal germinated seed, and five for percent viable firm swollen seed. These nine differences occurred in temperature regimes containing temperatures of ≤10°C. These temperature regimes were below the optimal germination conditions for corn (AOSA, 1998). Testing under suboptimal conditions can exacerbate small genetic differences. Isolated differences between MON 88017 corn and the control, with no concurrent trends across temperature regimes or production locations, are most likely due to random experimental error and not the result of altered germination characteristics of the seed.

These results are useful in establishing a basis to assess risk (including equivalence and familiarity). These results, and in particular, the lack of hard seed in MON 88017 corn, indicate that the genetic modification process, the presence of the coding sequence, or the gene products did not alter dormancy mechanisms in the seed. Thus, it is concluded that there was no change in the pest potential of MON 88017 from increased dormancy through hard seed.

VII.A.4. Phenotypic and Ecological Comparative Studies

Phenotypic and ecological data were collected to assess equivalence as it relates to pest potential and familiarity. A subset of the comparative phenotypic data (e.g., certain growth, reproductive, and pre-harvest seed loss characteristics) can be used for an assessment of enhanced weed potential of the modified crop.

Phenotypic data were collected from 18 field locations over two consecutive years: eight locations in 2001 and ten locations in 2002. Table VII-2 provides a list of locations and the corresponding USDA-APHIS notifications for each trial. These locations provided a range of environmental and agronomic conditions representative of major corn-growing regions where commercial production of MON 88017 corn would be expected.

Plots were established at each of the field sites in a randomized complete block design with four (2001 trials) or three (2002 trials) replications. Each plot consisted of two rows of corn spaced approximately 30 in. apart and approximately 17.5-20 ft in length.

The following 14 phenotypic characteristics were evaluated during 2001 and 2002: seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, staygreen, final stand count, dropped ears, stalk lodging, root lodging, test weight, grain moisture and yield. In addition, observational data on the presence of and differential response to biotic and abiotic stressors were collected. The observed stressors were not induced artificially; therefore, the same stressors were not observed at each field site. In the 2001 study, stressor monitoring was not documented at the JE, YK and TL sites. The evaluation and rating system is described in Table VII-1.

Phenotypic data were analyzed using Statistical Analysis Software (SAS). Means were calculated within-sites and across-sites for each characteristic, and MON 88017 was compared to the control using analysis of variance (ANOVA) methods. Differences were considered significant at the 5% level of significance ($p \leq 0.05$).

The following sections summarize the results from the two years of phenotypic and ecological evaluation of MON 88017. Results from individual sites and years are provided in Appendix F.

VII.A.4.a. 2001 Field Trials Results

Field trials were established at the eight locations listed in Table VII-2. The test and control materials used were positive (LH59 x MON 88017[+]/LH198BC1F_3) test and negative (LH59 x MON 88017[-]/LH198BC1F_3) control isolines, respectively, with the same genetic background (Figure V-12).

In 100 comparisons made between the test hybrid and control hybrid for the within-site analysis, only four statistically significant differences were noted: two at the TL site for ear height and grain moisture, one at the JV site for early stand count, and one at the FL site for staygreen (see Tables in Appendix F). The frequency of observed differences (4/100=4%) was below that expected due to random experimental effects (5%). Differences in these characteristics at single sites, with no consistent trend over sites, indicate that these differences are likely random experimental effects and unlikely to contribute to enhanced weed potential.

For all characteristics except seedling vigor, there were no statistically significant differences between the test and the control when averaged across-sites (Table VII-3). Test seedlings were more vigorous than those of the control. However, there were no concurrent across-site differences in stand count, days to pollen shed, or days to silk emergence. The impact of the difference in vigor would therefore seem unlikely to have a meaningful effect on plant biology and overall fitness with respect to altered weed potential of corn.

These results are useful in establishing a basis to assess risk including equivalence and familiarity. No differences in lodging or dropped ears between the test and control were observed, supporting a conclusion of no enhanced weed potential of MON 88017 corn. No differences among plots for responses to biotic and abiotic stressors were observed (Table VII-4), providing further information pertinent to the risk assessment.

Table VII-3. Phenotypic Comparison of MON 88017 to the Control Across All Sites in 2001 Field Trials

Characteristic[1]	MON 88017	Control
Seedling Vigor	3.7*	4.1*
Early Stand Count	69.1	66.8
Days to 50% Pollen Shed	66.8	66.6
Days to 50% Silk Emergence	66.9	66.9
Ear Height (in)	45.5	45.3
Plant Height (in)	88.8	87.9
Staygreen	5.5	5.7
Final Stand Count	61.6	63.3
Dropped Ears (#/plot)	0.5	0.7
Stalk Lodged Plants (#/plot)	5.4	4.1
Root Lodged Plants (#/plot)	0.7	0.6
Test Weight (lbs/bu)	54.5	54
Grain Moisture (%)	22.1	22
Yield (bu/a)	153.3	148

[1]Not all data was collected from all sites.

* Indicates that MON 88017 is statistically different from the control at $p \leq 0.05$.

Table VII-4. Summary of Ecological Interactions from 2001 Field Trials

Stressor	Sites	Range[1] observed in:	
		Test	Control
Insect			
Black cutworm	MN, RA	none-slt.	none-slt.
Corn earworm	MN, RA	none-slt.	none-slt.
Corn flea beetle	RA	none-slt.	none-slt.
Corn rootworm	FL, LA, MN, SC, RA	none-mod.	none-mod.
Corn rootworm beetle	LA, SC	none	none
Cutworm	LA, SC	none	none
Earworm	LA, SC	none	none
European corn borer	FL, LA, MN, SC, RA	none-mod.	none-mod.
Fall army worm	MN, RA	none	none
Grape colaspis	MN	none	none
Grasshoppers	RA	none	none
Seed corn maggots	MN, RA	none	none
White grubs	RA	none	none
Wireworms	RA	none	none
Disease			
Anthracnose stalk rot	LA, SC, RA	none-mod.	none-mod.
Aspergillus ear rot	RA	none	none
Common rust	FL, RA	none-slt.	none-slt.
Common smut	RA	none	none
Corn leaf rust	MN	none	none
Fusarium ear rot	RA	none	none
Gibberella stalk rot	RA	none	none
Gray leaf spot	FL, LA, MN, SC, RA	none-mod.	none-mod.
Northern corn leaf blight	LA, MN, SC	none	none
Penicillium kernel rot	RA	none	none
Seedling blight	RA	none	none
Southern corn leaf blight	LA, SC	none	none
Stalk rot	FL, MN	none	none
Stewart's wilt	LA, SC, RA	none-mod.	none-mod.
Abiotic			
Drought stress	LA, SC	none	none
Frost injury	RA	none	none
Hail damage	RA	none	none
Heat stress	LA, MN, SC, RA	none-slt.	none-slt.
Water stress	MN, RA	none	none
Wind damage	RA	none-mod.	none-mod.

[1]slt. = slight; mod. = moderate.

VII.A.4.b. 2002 Field Trial Results

Field trials were established at the ten locations listed in Table VII-2. The test and control materials were positive (LH59 x MON 88017[+]/LH198BC3F_3) and negative (LH59 x MON 88017[-]/LH198BC3F_3) isolines, respectively, having the same genetic background (Figure V-12).

Results from the within-site analysis of the phenotypic data showed that significant differences were observed for 11 of 140 (11/140 = 7.9%) comparisons made between MON 88017 and the control (see tables in Appendix F). This frequency was slightly above the rate expected due to random experimental effects alone (5%). When the data were pooled across all ten field sites, two differences were detected between MON 88017 and the control among the measured characteristics (Table VII-5). Seedling vigor for MON 88017 was greater compared to the control (7.6 vs. 6.5, respectively). Seedling vigor was assessed qualitatively on a scale of 0-9; therefore, the relatively small difference detected in seedling vigor across sites likely is not biologically meaningful with respect to plant weed potential. The number of days to 50% pollen shed was fewer for MON 88017 compared to the control (58.8 vs. 59.2 days, respectively). This difference was less than one day at most sites and it also is not likely to be biologically meaningful with respect to plant weed potential.

In the within-site analysis, seedling vigor was significantly greater for MON 88017 compared to the control at three of the ten sites and numerically greater at eight of the ten sites (Appendix F). This observed increase in seedling vigor was not manifested in other characteristics of growth, fitness, and reproduction, such as the number of days to 50% pollen shed, the number of days to 50% silking, plant height, or yield. The differences detected for seedling vigor at the three sites contributed largely to the difference detected across sites and were not indicative necessarily of a trend in the data. Seedling vigor was assessed qualitatively on a scale of 0-9; therefore, the small difference detected in seedling vigor across sites likely is not biologically meaningful with respect to weed potential. A consistent trend toward fewer days to 50% pollen shed would also not contribute to increased weed potential. In the within-site analysis, 50% pollen shed occurred one day earlier for MON 88017 compared to the control at one of the ten sites, and no statistically significant differences were detected at the other nine sites. The difference detected at the single site contributed largely to the difference detected across sites and was not indicative necessarily of a trend in the data.

Table VII-6 summarizes the ecological interaction observations from the 2002 field trials across all sites. Each field site was rated for specific insect pests, diseases, and abiotic stressors although not all sites were rated for each pest or stressor. Each plot was assessed qualitatively and assigned a numerical value of 0-9 based on rating scales for insect, disease, and abiotic stressors. A mean was calculated for each pest or stressor rating among the three replications per site and the numerical mean value was converted to a categorical value (i.e., none, slight, moderate, or severe) for reporting. If more than one site assessed a specific characteristic, the incidence reported corresponds to the mean numerical rating (0-9) calculated across sites. The significance of each ecological interaction characteristic was assessed by the expert opinion of the investigator.

Qualitative differences were observed between MON 88017 and the control for corn rootworm, anthracnose, and chemical injury incidence, each at a single site. Each observed qualitative difference was between consecutive rating categories (e.g., none slight vs. none moderate). Since there were no trends in susceptibility or tolerance to the observed pests and stressors across sites, a more quantitative assessment was not warranted. Differences noted in the table were likely an artifact of the assessment method (i.e., qualitative assessment of spatially variable pests and stressors among replications) and do not indicate necessarily a biologically meaningful result with respect to its impact on weed potential of the crop. These results support the conclusion that the ecological interactions for MON 88017 do not appear to have been altered by genetic modification.

The results of the phenotypic and ecological evaluations in 2002 show that most of the differences observed were for one or two characteristics at a single site, and not consistently across sites, leading to the conclusion that the genetic modification produced no meaningful impacts on the biological characteristics measured.

VII.A.5. Conclusions

Evaluation of 14 phenotypic characteristics of MON 88017 with that of control corn at 18 locations over two years showed no consistent trends other than for seedling vigor. All phenotypic characteristic data for which there were no detected differences between MON 88017 and the control support a conclusion of phenotypic equivalence as it relates to familiarity and a lack of increased weed potential. The statistically significant differences observed for seedling vigor in 2001 and 2002 were small in magnitude and not accompanied by consistent across-site differences in stand count, days to pollen shed, or days to silk. This suggests that it is unlikely that the differences observed for seedling vigor are biologically meaningful. The phenotypic data indicate that MON 88017 does not confer any detectable selective advantage to corn that would result in increased weed potential, compared to the control.

The ecological interaction data also indicate that MON 88017 does not confer any detectable increase in pest potential to corn, nor were there any detectable changes in the interactions between MON 88017 and the likely receiving environment. These conclusions were supported by the lack of detectable trends for differences in susceptibility or tolerance to specific insect, disease, or abiotic stressors across sites.

Table VII-5. Phenotypic Comparison of MON 88017 to the Control Across All Sites in 2002

Phenotypic characteristic		
	MON 88017	**Control**
Seedling vigor	7.6*†	6.5
Early stand count	64.1	61.9
Days to 50% pollen shed	58.8*†	59.2
Days to 50% silking	59.5	59.8
Stay green	3.8	3.9
Ear height (in)	40.5	41.4
Plant height (in)	82.2	82.8
Dropped ears (#/plot)	0.5	0.4
Stalk lodged plants (#/plot)	2.5	3.4
Root lodged plants (#/plot)	3.9	4.1
Final stand count	53.8	53.0
Grain moisture (%)	19.0	19.4
Test weight (lb/bu)	54.9	54.6
Yield (bu/a)	150.2	143.4

*Indicates a statistically significant difference between MON 88017 and the control at $p \leq 0.05$.
† Indicates a statistically significant interaction between MON 88017 and the control at $p \leq 0.05$.

Table VII-6. Summary of Ecological Interactions from 2002 Field Trials

Stressor	Sites[1]	Test	Control
Insect			
Black cutworm	BE, CR, MN, VH, WY	Slight	Slight
Corn rootworm	MN	Slight	None
Flea beetle	CL, CR	None	None
White grub	MN	Slight	Slight
Wireworm	CR	None	None
Disease			
Anthracnose	MN	None	Slight
Maize dwarf mosaic virus	CL	None	None
Ear rot	All ten sites	Slight	Slight
Leaf rust	CR	Slight	Slight
Leaf spot	BE, CL, CR, NB	Slight	Slight
Northern corn leaf blight	CL	Moderate	Moderate
Penicillium	CR, MN	Slight	Slight
Pythium	MN	Slight	Slight
Rhizoctonia	CR	None	None
Seedling blight	BE, CR	Slight	Slight
Southern corn leaf blight	RL	None	None
Stalk rot	All ten sites	Slight	Slight
Abiotic stressor			
Chemical injury	CL	Severe	Moderate
Chlorosis	NB	Slight	Slight
Cold	CR	None	None
Compaction	WY	Moderate	Moderate
Crusting	BE, CL	Moderate	Moderate
Drought	BE, CR	Severe	Severe
Flood	CR	None	None
Heat	CL, RL, WY, YK	Moderate	Moderate
Poor emergence	NB, VH	Slight	Slight
Stunting	BE	Slight	Slight
Wind	RL, VH	Moderate	Moderate

[1] Sites that were rated for specific insect, disease, or abiotic stressor. Note that not all sites were rated for each pest or stressor. If more than one site is listed, the incidence corresponds to the mean numerical rating across sites.

VII.A.6. Confirmatory Ecological Observations

Ecological stressor observations were recorded in test, control and reference substance production trials conducted at four locations in the U.S. during 2002 (see Table VII-2 for field locations). These trials were conducted to produce corn tissues for the estimation of CP4 EPSPS and Cry3Bb1 protein levels and compositional analysis. Plants were grown under agronomic and cultural practices that are typical of corn production. The field plots were monitored approximately every month and observed for plant stressors, including susceptibility to common insect pests and pathogens (see Table F-19 in Appendix F). Slight to moderate effects were noted from heat and drought stress. The only diseases observed were common rust and anthracnose, each at one location. Observations of a few insect species were noted, including grasshoppers, spider mites and corn rootworms. There were no trends indicating differences between the test and control plots, except for a single occurrence of corn rootworm in control plots but not in test plots at the Nebraska location. This difference was not unexpected because of the presence of the corn rootworm-protection trait in MON 88017.

Ecological observations were also recorded in product evaluation field trials conducted during 2000-2003 under USDA notification (see Appendix G for a listing and status of field trial final reports). The trials were conducted under various product development protocols including: breeding and observation nursery, inbred seed production, line *per se* and yield, insect efficacy, glyphosate tolerance, product characterization and performance, insect resistance management, and nontarget organism (NTO) evaluation. Collectively, the data from these trials show no significant differences in insect and disease susceptibility between MON 88017 and the conventional control, except for tolerance to the application of Roundup agricultural herbicides and resistance to corn rootworm larval feeding damage.

VII.A.7. Pollen Morphology and Viability

The purpose of this evaluation was to assess whether the presence of the *cp4 epsps* and *cry3Bb1* genes, the presence of the gene products (CP4 EPSPS and Cry3Bb1 proteins), or the genetic modification process altered the pollen characteristics of MON 88017 compared to the control. The test substance was corn pollen collected from MON 88017 plants. The control substance was corn pollen collected from conventional corn plants with a genetic background similar to MON 88017. The reference substances were pollen collected from plants of commercially available conventional corn hybrids and were used to demonstrate a range of morphology and viability characteristics for corn pollen. Pollen samples were collected from a field trial conducted in Jersey County, IL in 2003 (USDA notification 03-112-14n).

The plots were arranged in a completely randomized design with no replication (a single plot for each test, control, and reference substance). Pollen was collected from 15 individual corn plants selected at random from the plants pollinating within each plot. The samples were stained and viewed microscopically. Pollen viability was determined for each of the 15 samples per substance under 70X magnification. Pollen morphology

was evaluated for three samples per substance by measuring the diameter of ten viable pollen grains under 100X magnification. Variance analysis was conducted according to a completely randomized design and the test substance was compared to the control substance for average pollen grain diameter and percent viable pollen. Any differences were considered significant at a level of $p \leq 0.05$.

Results showed no statistically significant differences between MON 88017 and the control for average pollen diameter. The pollen from MON 88017 had statistically significant greater viability than the control (99.4% vs. 98.0%), but was within the range of values observed in the reference substances (86-100%) (Table VII-7). Corn produces abundant pollen and viability can range from 0% in male sterile corn to 100% (as observed in this study); therefore, this difference (1.4%) was considered small and unlikely to be of biological significance.

Observational comparisons of the pollen samples were made using a microscope. No visual differences in the overall morphology of the pollen samples were visually noted between MON 88017 and the control for viable pollen grains. Representative micrographs of pollen from MON 88017 and the control are presented in Figures VII-1 and VII-2.

These results demonstrated that the presence of the *cp4 epsps* and *cry3Bb1* genes, the presence of the gene products (CP4 EPSPS and Cry3Bb1 proteins), or the genetic modification process did not alter the overall morphology or viability of pollen from MON 88017 compared to the control.

Table VII-7. Comparison of MON 88017 and Control Pollen

Substance	Average Pollen Diameter (µm)[1]	Viable Pollen (%)[2]
MON 88017	78.8	99.4*
Control	77.0	98.0
Reference Range[3]	60-95	86-100

* Indicates the test substance value is statistically significantly different than the control substance value at $p \leq 0.05$.
[1] Number of samples = 3.
[2] Number of samples = 15.
[3] Range of values observed among the four reference pollen samples.

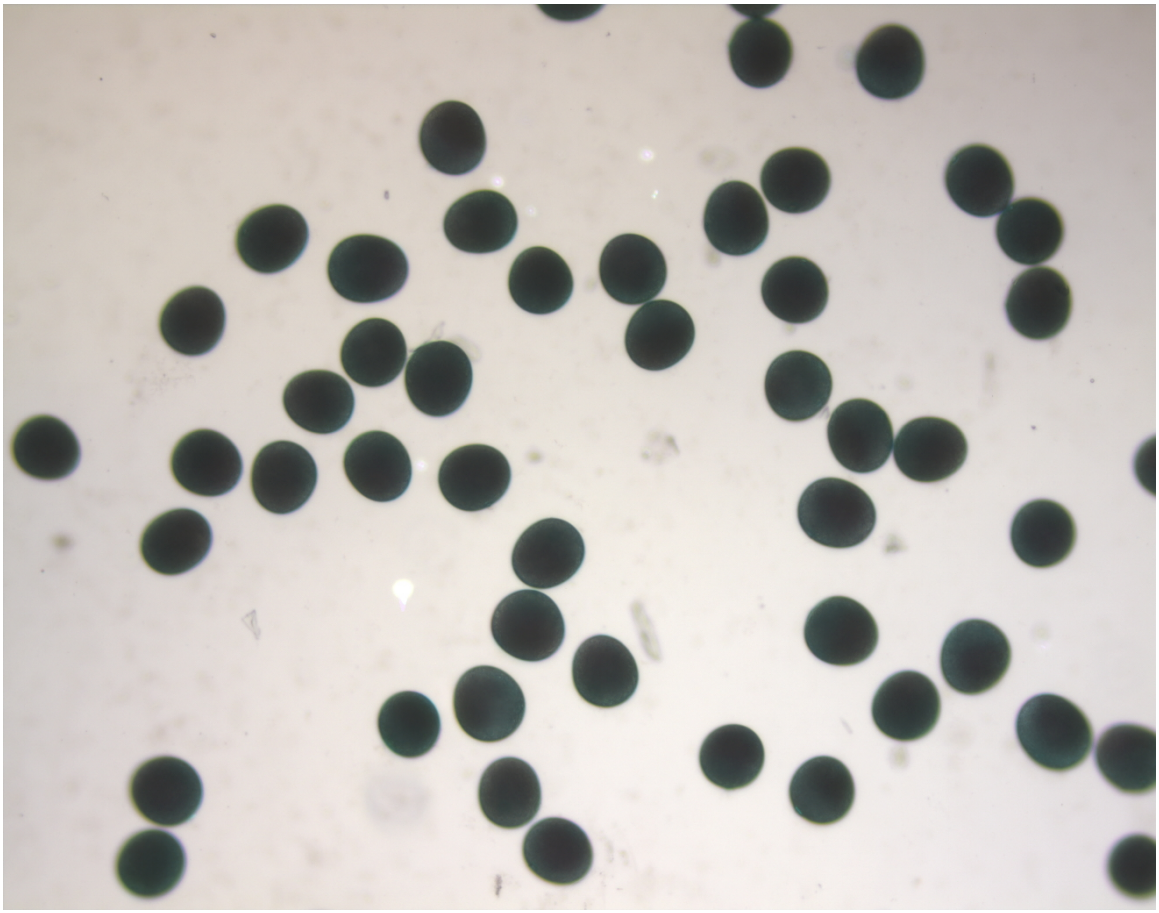


Figure VII-1. Photograph of MON 88017 Pollen at 100X Magnification

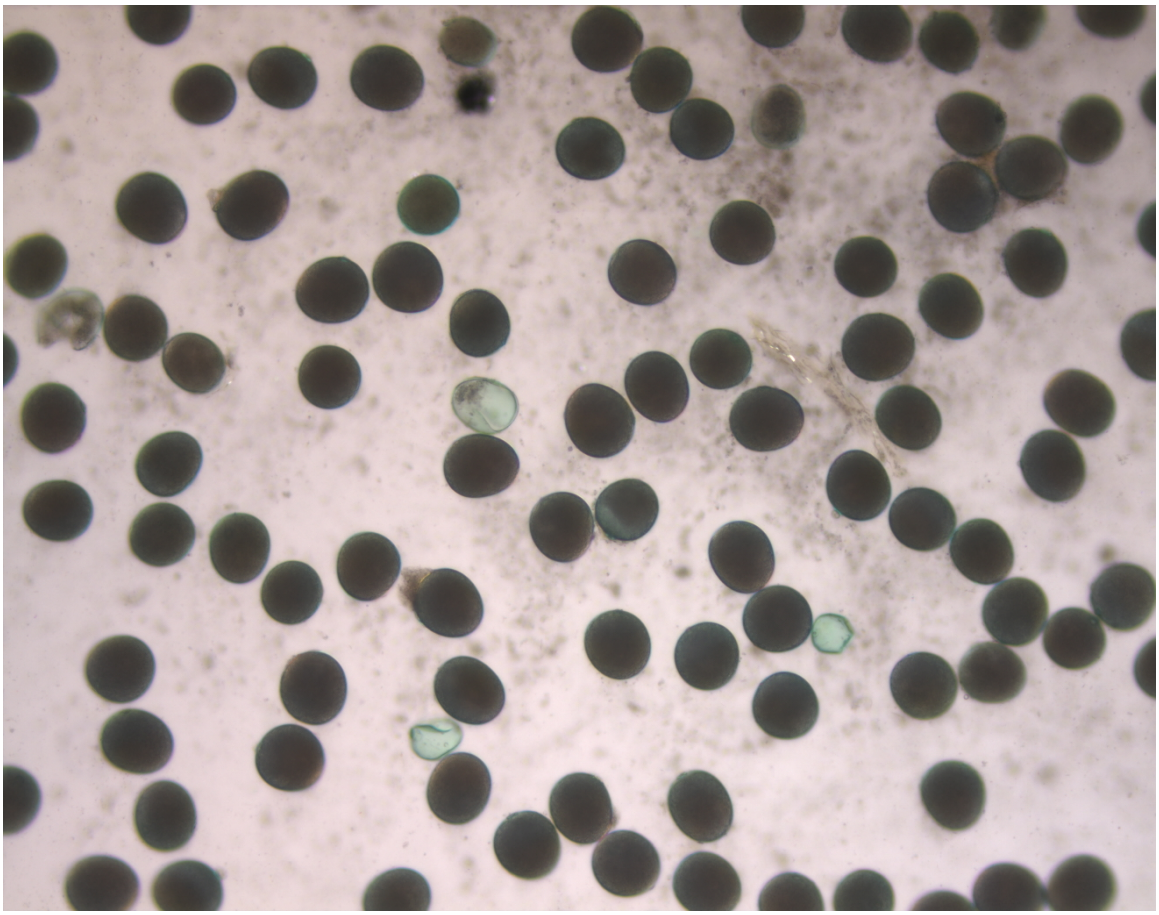


Figure VII-2. Photograph of Control Pollen at 100X Magnification

VII.A.8. Conclusion of the Phenotypic and Ecological Evaluations

Data developed from these investigations represent a broad range of environmental conditions and agronomic practices that MON 88017 likely would encounter. These data represent observations that typically are recorded by plant breeders and agronomists to evaluate the qualities of corn. The characteristics measured provide crop biology data useful in establishing a basis to assess equivalence and familiarity in the context of ecological risk assessment. The phenotypic characteristic data showed no biologically meaningful differences between MON 88017, control and conventional reference corn and support a conclusion of phenotypic equivalence as it relates to familiarity and a lack of increased weed potential. Detected differences were considered alone, in consideration of other observed differences, and for trends across locations. Each detected difference was considered with respect to its impact for increased weed potential of the crop itself and if the trait were transferred to a wild relative. The phenotypic data indicate that MON 88017 does not confer a selective advantage to corn that would result in increased weed potential.

VII.B. Composition of MON 88017

The composition of forage and grain produced by MON 88017 was evaluated and compared to a conventional control corn with similar genetic background, as well as other commercially available corn hybrids. MON 88017 and the conventional control corn were grown at three replicated field sites in major corn-growing areas of the U.S. (Iowa, Illinois and Nebraska) during the 2002 field season. Four commercially available corn hybrids were grown also at each of the same field sites to provide a total of 12 different reference substances. At each field site, the test, control and reference seed were planted in a randomized complete block design with three replicates per block. All the plants were grown under normal agronomic field conditions for their respective geographic regions. All test plots received an application of Roundup UltraMAX herbicide according to label directions. Bulk forage samples were harvested at the late dough/early dent stage, and bulk grain samples were harvested when the grain was mature.

Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium, phosphorus), and carbohydrates by calculation. Compositional analyses of the grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), and carbohydrates by calculation. In all, 77 different analytical components (nine in forage and 68 in grain) were analyzed. The following 15 compositional analytes with >50% of observations below the limit of quantitation (LOQ) of the assay were excluded from statistical analysis: sodium, 2-furaldehyde, 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic

acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. Therefore, 62 components were statistically assessed (nine in forage and 53 in grain) for the compositional equivalence of MON 88017.

Statistical analyses of the compositional data were conducted using a mixed model analysis of variance method. Four sets of analyses were made based on data from each of the three replicated field sites plus data from a combination of all three field sites. Statistical evaluation of the composition data involved comparison of the forage and grain from the test substance to the control. Statistically significant differences were determined at the 5% level of significance ($p<0.05$). There were 248 comparisons (four sets of analyses x 62 components assessed) conducted on the test hybrid. Using the data for each component obtained from the 12 different commercial hybrids, a 99% tolerance interval was calculated to contain, with 95% confidence, 99% of the values contained in the population of commercial corn. For those comparisons in which the test was statistically different from the control, the test range was compared to the 99% tolerance interval in order to determine if the test range was within the interval and therefore considered to be part of the population of the commercial corn. Table VII-8 presents a summary of the statistically significant differences and Appendix H provides the compositional values for the analysis across sites.

Results of the forage and grain sample analysis showed that there were no statistically significant differences ($p<0.05$) between MON 88017 and the conventional control for 232 of the 248 comparisons conducted. There were no statistically significant differences found in forage. For grain, statistically significant differences were observed for the content of: 16:0 palmitic acid, 18:1 oleic acid, 18:3 linolenic acid, 20:0 arachidic acid, copper, methionine, moisture, niacin, and serine (one comparison each); 18:2 linoleic acid (three comparisons); and vitamin B_1 (four comparisons). Five percent, or approximately 12 (0.05×248) comparisons, were expected to be statistically significant based upon chance alone. Except for vitamin B_1, none of the statistically significant differences were in all four analyses (each individual site and the combination of all sites). The vitamin B_1 values in grain were statistically lower in MON 88017 compared to conventional corn for all four analyses, but they were similar to literature and historical values of vitamin B_1 in corn grain. All test values were also within the 99% tolerance interval for the 16 comparisons observed to be statistically different between MON 88017 and the conventional control. Therefore it is unlikely that these differences are biologically meaningful.

Based on these data, it is concluded that the forage and grain produced from MON 88017 are compositionally equivalent to the forage and grain produced from corn currently on the market.

Table VII-8. Summary of the Statistical Differences for the Compositional Comparison of MON 88017 to Control Corn

Tissue/Site/ Component (Units)[a]	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval[b]
Grain						
IA						
16:0 palmitic (% total FA)	10.16	12.94	-21.50	0.029	(10.11-10.23)	[6.51, 16.50]
18:2 linoleic (% total FA)	63.25	60.41	4.70	0.017	(62.73-63.72)	[41.22, 74.09]
18:3 linolenic (% total FA)	1.25	1.57	-20.26	0.036	(1.24-1.26)	[0.42, 1.95]
Methionine (% total AA)	2.02	2.16	-6.39	<0.001	(1.96-2.05)	[1.37, 2.60]
Moisture (% fw)	9.38	9.93	-5.54	0.034	(9.03-9.70)	[4.67, 17.56]
Vitamin B_1 (mg/kg dwt)	2.54	3.07	-17.37	<0.001	(2.42-2.65)	[1.96, 4.38]
IL						
18:1 oleic (% total FA)	22.53	23.29	-3.26	<0.001	(22.50-22.56)	[9.25, 44.14]
18:2 linoleic (% total FA)	63.11	62.15	1.55	0.003	(62.84-63.29)	[41.22, 74.09]
Niacin (mg/kg dwt)	21.10	22.52	-6.30	0.014	(20.39-21.52)	[3.19, 34.49]
Vitamin B_1 (mg/kg dwt)	2.30	3.10	-25.63	<0.001	(2.30-2.30)	[1.96, 4.38]
NE						
Copper (mg/kg dwt)	1.57	2.21	-28.80	0.023	(1.48-1.68)	[0.17, 3.00]
Serine (% total AA)	4.80	4.97	-3.37	0.042	(4.80-4.81)	[4.60, 5.43]
Vitamin B_1 (mg/kg dwt)	2.58	3.56	-27.53	<0.001	(2.47-2.69)	[1.96, 4.38]

Table VII-8 (cont'd). Summary of the Statistical Differences for the Comparison of MON 88017 to Control Corn

Tissue/Site/ Component (Units)[a]	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval[b]
Grain						
Combination of all sites						
18:2 linoleic (% total FA)	62.85	61.52	2.17	0.038	(61.86-63.72)	[41.22, 74.09]
20:0 arachidic (% total FA)	0.37	0.38	-2.24	0.012	(0.35-0.39)	[0.31, 0.49]
Vitamin B_1 (mg/kg dwt)	2.47	3.24	-23.72	<0.001	(2.30-2.69)	[1.96, 4.38]

[a]dwt=dry weight; fw=fresh weight; AA=amino acids; FA=fatty acids
[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

VII.C. Overall Conclusions for Phenotypic, Ecological and Compositional Assessment of MON 88017

Information was used to assess whether the presence of the introduced CP4 EPSPS and Cry3Bb1 proteins altered the plant pest characteristics of MON 88017 compared to the control. The assessment was based on a thorough phenotypic, ecological and compositional characterization and comparison of MON 88017 to control and conventional reference corn. Data was collected for five seed dormancy/germination parameters, 14 phenotypic characteristics during plant growth and development, two reproductive morphology characteristics, and 77 compositional components. In addition, ecological observational data were collected for the presence of, and any differential response to, biotic (pests and disease) and abiotic stressors. These measurements are well known to corn breeders and can provide supplementary data to assess plant pest potential.

The characteristics measured provide crop biology data useful in assessing equivalence and familiarity in the context of ecological risk assessment. All phenotypic characteristic data for which there were no detected differences between MON 88017 and the control support a conclusion of phenotypic equivalence as it relates to familiarity and a lack of increased weed potential. Detected differences were evaluated alone, in consideration of other observed differences, and for trends across sites. Each detected difference was considered with respect to its impact for increased weed potential of the crop itself and if the trait were transferred to a wild relative.

The overall conclusions from this assessment were that there are no biologically meaningful differences in terms of pest potential between MON 88017 and control or conventional corn.

VIII. Enviromental Assessment and Impact on Agronomic Practices

This section provides an environmental assessment of MON 88017 including the impact of the introduced CP4 EPSPS and Cry3Bb1 proteins, the potential for gene flow, and weediness potential of MON 88017. The section also provides a summary of current agronomic practices for corn and an assessment of the impact from the introduction of MON 88017 on crop rotational practices, the management of volunteers, and weed and insect resistance management.

VIII.A. Environmental Assessment of the CP4 EPSPS Protein

VIII.A.1. Safety of the Introduced Trait

Five Roundup Ready crops that produce the CP4 EPSPS protein have been reviewed by regulatory agencies and cleared for environmental release in one or more countries around the world. The products are Roundup Ready corn, canola, cotton, soybean, and sugar beet. Since the introduction of Roundup Ready soybean in the U.S. in 1996, growers have used these products with no reports of negative effects on nonpest organisms and the environment. Furthermore, extensive compositional data have demonstrated that these crops are nutritionally equivalent to their conventional counterparts. The safety assessment of the CP4 EPSPS protein produced in MON 88017 includes a protein characterization demonstrating the lack of similarity to known allergens and toxins and the long history of safe consumption of similar proteins. In addition, data confirm the CP4 EPSPS protein digestibility *in vitro*, and the lack of acute oral toxicity in mice.

VIII.A.2. Effects on Pest and Nonpest Species

The CP4 EPSPS protein produced in MON 88017 and other Roundup Ready crops is similar to the native EPSPS proteins that are ubiquitous in plant and microbial tissues in the environment. Therefore, based on this history of occurrence, the EPSPS protein is not expected to possess biological activity towards nonpest organisms. Even though the likelihood of hazard is low for the CP4 EPSPS protein, a number of researchers have conducted laboratory investigations with different types of arthropods exposed to Roundup Ready crops (Goldstein, 2003; Boongird et al., 2003; Jamornman et al., 2003; Harvey et al., 2003). Representative pollinators, soil organisms, beneficial arthropods, and pest species were exposed to tissues (pollen, seed, and foliage) from Roundup Ready crops. These studies, although varying in design, all reported a lack of toxicity observed in various species exposed to Roundup Ready crops producing the CP4 EPSPS protein (Nahas et al., 2001; Dunfield and Germida 2003, Siciliano and Germida, 1999).

The lack of toxicity is further supported by field experimentation conducted on biotechnology-derived crops producing the CP4 EPSPS protein. The diversity and abundance of Collembola were no different between Roundup Ready soybeans and conventional soybeans grown under the same management systems (Bitzer et al., 2000). Other studies on Roundup Ready soybeans under various weed management systems

concluded that there was no apparent direct effect of the Roundup Ready trait on arthropods, although weed management and phenotypic differences (plant height or maturity) associated with plant variety influenced arthropod populations (Jasinski et al., 2003; McPherson et al., 2003; Buckelew et al., 2000). A similar lack of effect on arthropods is expected for MON 88017.

VIII.B. Environmental Assessment of the Cry3Bb1 Protein

The USDA and EPA have previously conducted environmental assessments of the Cry3Bb1 protein produced in YieldGard Rootworm corn (MON 863).

In the environmental assessment of MON 863 (Petition 01-137-01p), USDA-APHIS considered the potential impact of the Cry3Bb1 protein on NTOs, including beneficial organisms and threatened or endangered species. The USDA concluded: "It [MON 863] has no potential to have a greater damaging, harmful, or toxic effect on organisms beneficial to agriculture than does other cultivated corn. In addition to our finding of no plant pest risk, there will be no effect on threatened or endangered species resulting from a determination of non-regulated status for MON 863 corn and its progeny."

The EPA conducted an extensive environmental hazard assessment of Cry3Bb1-producing corn based on studies conducted with several Cry3Bb1 protein variants on representative species of bird, fish, and terrestrial nontarget insects to support the registration of MON 863 (EPA, 2003). The EPA also considered possible effects on endangered species and the fate of the Cry3Bb1 protein in the environment. Recommendations from the FIFRA Scientific Advisory Panel (SAP) meeting (EPA, 2002), consultations with scientific experts, and public comments received by the EPA were also considered. The EPA concluded, "The Agency has sufficient information to believe that there is no risk from the proposed uses of Cry3Bb1 corn to non-target wildlife, aquatic and soil organisms. However, after consultation with the FIFRA SAP in August 2002 and from several public comments, the Agency is requesting additional data. The supplementary studies would provide additional weight to the Agency's conclusions." Monsanto will submit the supplementary information requested by the EPA as it becomes available.

Because MON 88017 contains a Cry3Bb1 protein that is physicochemically and functionally equivalent to the Cry3Bb1 protein produced in MON 863, the conclusion of no risk to the environment should apply equally to this protein. The additional data requested by the EPA as a condition of the registration for MON 863 will also support a determination of no risk to the environment for MON 88017. A justification for this conclusion is provided below.

VIII.B.1. Activity Against Target Insects

The Cry3Bb1 protein has natural insecticidal activity against the coleopteran pest, corn rootworm (Von Tersch et al., 1994). Monsanto has conducted a series of dietary bioassays to characterize the insecticidal spectrum of activity of Cry3Bb1 that supports the conclusion that the insecticidal activity of Cry3Bb1 is most evident in beetle species

within the family Chrysomelidae (EPA, 2003). As shown previously (Section VI.B.1), the Cry3Bb1 protein produced in MON 88017 has activity against the Coleopteran pest, Colorado potato beetle (CPB). Additional laboratory and field evaluations (described below) show that the insecticidal activity of the Cry3Bb1 protein produced in MON 88017 is equivalent to that of the Cry3Bb1 protein produced in MON 863.

VIII.B.1.a. Functional Activity of the Cry3Bb1 Proteins Produced in MON 88017 and MON 863

The functional equivalence of the Cry3Bb1 protein produced in MON 88017 and the Cry3Bb1 protein produced in MON 863 was evaluated by comparing biological activity against two susceptible species of insects, larvae of CPB and larvae of WCRW. Diet-incorporation insect bioassays were used to determine the biological activity of the two Cry3Bb1 protein variants against each of the test coleopteran insects. Data from these bioassays, conducted in triplicate for each combination of target insect and Cry3Bb1 protein variant, were analyzed with probit models for estimation of the LC_{50} values (i.e., lethal concentrations required to kill 50% of the test insect larvae).

Results from the LC_{50} estimation showed that both Cry3Bb1 protein variants have similar biological activity against larvae of CPB as well as against larvae of WCRW. The mean LC_{50} values were 0.84 μg/ml and 0.95 μg/ml of the diet for for CPB larvae, and 139 μg/ml and 100 μg/ml of the diet for WCRW larvae, for the Cry3Bb1 proteins produced in MON 88017 and MON 863, respectively.

Integrated probit analysis of the bioassay data on each of the two insect species indicated that there were no significant differences in biological activity between the two Cry3Bb1 protein variants against either CPB ($p = 0.1262$) or WCRW larvae ($p = 0.2443$). This leads to the conclusion that the Cry3Bb1 proteins produced in MON 88017 and MON 863 are functionally equivalent in biological activity against the susceptible coleopteran insect species, CPB and WCRW.

VIII.B.1.b. Field Efficacy of MON 88017 and MON 863

The field efficacy of MON 863 and MON 88017 against WCRW larvae was evaluated at 12 locations in the U.S during 2002 and 2003. The 2002 field trials were conducted at seven locations in Iowa, Illinois, Nebraska, and South Dakota and the 2003 field trials were conducted at five locations in Iowa and Illinois.[3] The experimental design consisted of a randomized complete block with four replications each of MON 88017, MON 863, and conventional control corn. Single-row plots ranged from 17.5 to 20 feet in length with 30 seeds planted per row. All experiments were bordered by four rows of commercial MON 810 corn. Each row was artificially infested with approximately 1400 to 1600 WCRW eggs/row-ft. Infestation rates and procedures were based on the past research by Branson et al. (1980) and Sutter and Branson (1980), respectively.

[3] The USDA notifications and counties for the trials in 2002 were as follows: 02-023-02n for the counties of Story, Cass, and Linn, IA; 02-023-03n for the counties of Jersey and Warren, IL; 02-010-15n for Brookings, SD; and 02-092-07n for Henderson, NE. For the trials in 2003, the USDA notifications were: 03-030-09n for the counties of Story and Linn, IA; and 03-030-08n for the counties of Warren-1, Warren-2 and Hancock, IL.

Infestations were completed at the V2 (second leaf) corn growth stage at all sites. Each location followed recommended agricultural production practices, such as fertility and weed control.

Approximately eight to ten roots per replication were excavated prior to the VT (tasseling) corn growth stage at approximately six to seven weeks following egg inoculation. Soil was removed from roots so they could be evaluated for feeding damage using the Oelson Node Injury Scale (Oelson and Tollefson, 2001). All root damage ratings (RDR) data were subjected to statistical analysis using analysis of variance (ANOVA) methods. A Tukey-Kramer Honestly Significant Difference (HSD) test was then used to compare differences between all hybrids while controlling for the experiment-wise error rate of 0.05 (SAS, 2000).

Individual site analysis from the 2002 and 2003 efficacy trials indicated that all 12 sites had moderate to high pressure; the average RDR for the conventional control was 1.47 and ranged from 0.81 to 2.38. Analysis of variance showed a significant site effect ($P>F$ <0.0001), which is expected because of the varying levels of pressure found on the control corn. However, at each location, MON 88017 and MON 863 were found to be statistically similar, and each controlled corn rootworm larval feeding statistically better than the control. Across sites, MON 88017 and MON 863 had an average RDR of 0.12 and were not statistically different from one another.

These results lead to the conclusion that the Cry3Bb1 protein produced in MON 88017 provided significant control against corn rootworm larval feeding that is equivalent to that provided by the Cry3Bb1 protein produced in MON 863.

VIII.B.2. Impact on NTOs

Potential adverse effects to NTOs resulting from exposure to Cry3Bb1 protein variants have been previously evaluated in a series of studies with representative avian (bobwhite quail), aquatic (catfish and water fleas), and terrestrial beneficial invertebrate species (Collembola, ladybird beetle, adult and larval honey bees, green lacewing, parastic wasp, and earthworm). These NTOs were exposed to high doses of corn leaf, grain or pollen tissue containing a plant-produced Cry3Bb1 variant, or to an artificial diet containing a *B.t.- or E. coli*-produced Cry3Bb1 variant. In each case a no-observed-effect concentration (NOEC) for the Cry3Bb1 protein variant was established. Margins of exposure (MOEs) were calculated based on the ratio of the NOECs to the maximum predicted environmental concentration (MEEC). Results showed that the MOEs ranged from three- to 141-fold (EPA, 2003). Further, feeding corn grain containing Cry3Bb1 protein variants to bobwhite quail and channel catfish at 10 and 35 wt% of their diets, respectively, resulted in no adverse effects on growth or survival. These results indicate that the Cry3Bb1 protein variants do not pose significant risks to NTOs.

A theoretical estimate of the MOEs for the Cry3Bb1 protein produced in MON 88017 can be calculated for certain NTOs based on the NOEC values reported previously (EPA, 2003), and MEECs calculated based on the expression of the Cry3Bb1 protein produced

in MON 88017 in various tissues.[4] The MEEC for organisms feeding on corn plants is predicted to be 20 μg/g fwt and 110 μg/g fwt based on the highest level of Cry3Bb1 found in pollen and leaf tissue of MON 88017, respectively (see Tables VI-1 and VI-3). The MEEC for soil-dwelling organisms is predicted to be 9.85 mg/kg based on the assumption that 25,000 plants/acre are tilled into the top six inches of soil at the time of maximum Cry3Bb1 concentration in plants (i.e., 110 μg/g fwt). The use of the soil MEEC to calculate MOEs for Collembola is appropriate because some Collembola feeding on detritus may inadvertently consume soil as well. Some Collembola, however, can consume plant tissue directly and the tissue MEEC may be a better measure of the MOE. In this case, the MOE for MON 88017 is calculated to be 7.9, still indicating minimal risk to Collembola. The MEEC for aquatic organisms is predicted to be 0.0034 μg/l based on the following assumptions: the pollen concentration of Cry3Bb1 is 20 μg/g fwt, the edge of field deposition rate for pollen is 0.02-0.03 mg/cm^2, and the pollen drifts into a body of water 2 m deep.

Results (Table VIII-1) show that the estimated MOEs for NTOs exposed to the Cry3Bb1 protein produced in MON 88017 are generally similar to or greater than those observed for the Cry3Bb1 protein produced in MON 863, with the differences attributed to differences in Cry3Bb1 protein expression in leaf and pollen tissues. These results demonstrate that the overall conclusion of no adverse risk to NTOs reached for the Cry3Bb1 protein produced in MON 863 should apply equally to the Cry3Bb1 protein produced in MON 88017.

The Cry3Bb1 protein produced in MON 88017 is unlikely to have any impact on field abundance of NTOs, based on the results of a two-year field study conducted for the Cry3Bb1 protein produced in MON 863. The abundance of prominent beneficial nontarget invertebrate species was found to be comparable for plots planted with either conventional corn or MON 863 (EPA, 2003). In some cases, the abundance of nontarget invertebrate species was higher in plots planted with MON 863 than in plots treated with insecticides. This likely is because of the narrow insecticidal spectrum of the Cry3Bb1 protein versus the broad spectrum of activity typical of most insecticides.

[4] Cry3Bb1 protein levels expressed in μg/g fwt instead of μg/g dwt were used for consistency with previous MEEC calculations conducted for MON 863.

Table VIII-1. Calculated Margins of Exposure to NTOs for the Cry3Bb1 Protein Produced in MON 88017[1]

				Margins of Exposure	
Test Organism	**Cry3Bb1 variant**	**Origin (Tissue)**	**Results[2]**	**MON 88017[3] (NOEC ≥)**	**MON 863[4] (NOEC ≥)**
Cladoceran (*Daphnia magna*)	11098 (Q349R)	MON 863 (pollen)	NOEC ≥ 2.26 µg/l	665x surface water MEEC	141x surface water MEEC
Collembola (*Folsomia candida*)	11098 (Q349R)	MON 863 (leaf)	NOEC ≥ 872.5 µg/g	88.6x soil MEEC	105x soil MEEC
Adult Honey Bee (*Apis mellifera*)	11231	*B.t.*	NOEC ≥ 360 µg/ml	18x max. pollen level	3.8x max. pollen level
Larval Honey Bee (*Apis mellifera*)	11231	*B.t.*	NOEC ≥ 1790 µg/ml as a single dose	89.5x max. pollen level	19x max. pollen level
Adult Ladybird Beetle (*Hippodamia convergens*)	11231	*B.t.*	NOEC ≥ 8000 µg/g	400x max. pollen level	86x max. pollen level
Green Lacewing Larvae (*Chrysoperla carnea*)	11231	*B.t.*	NOEC ≥ 8000 µg/g	400x max. pollen level	86x max. pollen level
Parasitic Hymenoptera (*Nasonia vitripennis*)	11231	*B.t.*	NOEC = 400 µg/ml	20x max. pollen level	4.3x max. pollen level
Earthworm (*Eisenia fetida*)	11231	*B.t.*	NOEC = 57 mg/kg	5.8x MEEC in soil	6.9x MEEC in soil

[1]NOEC = no observed effect concentration ; [c]MEEC = maximum expected environmental concentration ; Margin of exposure = ratio of NOEC to MEEC.

[2] EPA, 2003.

[3]Based on the following MEEC values for the Cry3Bb1 protein: 110 µg/g fwt in leaf, 20 µg/g fwt in pollen, 9.85 mg/kg in soil, and 0.0034 µg/l in aquatic environments.

[4]Values for Collembola and earthworm reported in EPA, 2003 were revised because of a calculation based on 25,000 vs. 40,000 plants/acre.

VIII.B.3. Impact on Endangered Species

As described above, Cry3Bb1 proteins are essentially nontoxic to non-insect species, thus they pose no significant risk to endangered mammals, birds, non-insect aquatic organisms and non-insect soil dwelling organisms. Only insects in the order Coleoptera (beetles) have been found to be sensitive to Cry3Bb1 protein, and this sensitivity has thus far been limited to beetles of the Chrysomelidae family. Because there are no endangered beetles in the Chrysomelidae family, there is no possibility of effects on these coleopterans. In the case of other coleopteran species, a review of the susceptibility profile compared to potential exposure levels in the field indicated that endangered species would not be adversely affected (EPA, 2003).

VIII.B.4. Dissipation in Soil and Impact on Soil-dwelling Microrganisms

Laboratory aerobic soil degradation studies demonstrate that the Cry3Bb1 protein dissipates rapidly in the environment. For example, when shoots and roots of MON 863 corn were incubated in three different soils, the DT_{50} and DT_{90} estimates for Cry3Bb1 protein were found to range from 0.9 to 2.3 days and from 7.4 to 50 days, respectively (EPA, 2003). In addition, rapid soil dissipation of the purified Cry3Bb1 protein in the three soils (DT_{90} = 4 to 5.2 days) suggests that any protein reaching the soil by root exudation, i.e., not combined with tissue, likely would be more than 90% degraded in less than six days. The Cry3Bb1 protein produced in MON 88017 is likely to show a similar soil degradation profile considering that the levels present in whole plant and roots are similar to those found for the Cry3Bb1 protein produced in MON 863.

While the effect of *B.t.* proteins on soil-dwelling organisms has not been studied extensively, recent literature reports suggest that there should be little to no impact on soil microflora from the use of plants producing *B.t.* Cry proteins. For example, in a season-long field study conducted with the Cry3A protein expressed in biotechnology-enhanced potato, no adverse effects towards soil-dwelling microrganisms were observed (Donegan et al., 1996). In a study conducted in Kansas during the 2000 and 2001 growing seasons, the numbers of soil mites, Collembola and nematodes observed in plots planted with Cry3Bb1-producing corn were similar to those observed in plots planted with conventional corn (Al-Deeb et al., 2003). A recent literature report showed that two *B.t.* proteins had no microbiocidal or microbiostatic activity *in vitro* against selected bacteria, fungi, and algae (Koskella and Stotzky, 2002). These results suggest that the risk posed by the exposure of the Cry3Bb1 protein to soil-dwelling organisms will be minimal.

VIII.B.5. Conclusion

The environmental assessment of the Cry3Bb1 protein produced in MON 88017 considered 1) the extensive database of ecological studies previously conducted for the Cry3Bb1 protein produced in MON 863; 2) its physicochemical and functional characterization; 3) equivalent efficacy to the Cry3Bb1 protein in MON 863 against target insects; 4) calculated MOEs for NTOs similar to those of the Cry3Bb1 protein produced in MON 863; and 5) expected lack of effects to endangered species and soil

microorganisms. The results of this assessment lead to the conclusion that the use of MON 88017 corn will pose no significant risk to the environment.

VIII.C. Weediness Potential of MON 88017

In the United States, corn is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979; Muenscher, 1980), nor is it present on the lists of noxious weed species distributed by the Federal Government (7 CFR Part 360). Furthermore, corn has been grown throughout the world without any report that it is a serious weed. 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 does not occur naturally because of the structure of ears of corn. Even if individual kernels of corn were distributed in the fields and main avenues of travel from the fields to storage facilities, volunteer corn is not found growing in fence rows, ditches, and road sides as a weed. Although corn seed can overwinter into a crop rotation with soybeans, mechanical and chemical measures can be used to control volunteers. Corn is poorly suited to survive without human assistance and is not capable of surviving as a weed (Baker, 1965; Keeler, 1989; Galinat, 1988).

In the comparative studies conducted between MON 88017 and a conventional control, dormancy, germination, phenotypic, and pollen morphology characteristics were evaluated for changes that would impact plant pest potential, and in particular, plant weed potential (see Section VII). The evaluation of these characteristics did not detect any biologically meaningful differences between MON 88017, control and conventional corn and support a conclusion of equivalence as it relates to familiarity and a lack of increased weed potential. Furthermore, extensive postharvest monitoring of field trial plots planted with MON 88017 under USDA-APHIS notifications did not reveal any differences in survivability or persistence relative to other corn. These data suggest that MON 88017 is no more likely to become a weed than conventional corn.

MON 88017 contains the identical CP4 EPSPS protein present in Roundup Ready corn NK603 that was commercialized in the U.S. in 2000. In the three years following commercial introduction, there have been no reports that corn with this trait has become a weed problem. MON 88017 also contains a Cry3Bb1 protein that is 99.8% identical in its amino acid sequence to that of the Cry3Bb1 protein present in commercial YieldGard Rootworm corn (MON 863). In the environmental assessment for the deregulation of YieldGard Rootworm corn MON 863 (Petition 01-137-01p), USDA-APHIS stated: “The introduced trait, coleopteran insect resistance, is not expected to cause MON 863 corn to become a weed.”

This evaluation leads to the conclusion that MON 88017 is no more likely to become a weed than conventional corn.

VIII.D. Gene Flow

Gene flow (often used synonymously with the term "outcrossing") is a natural biological process that occurs in most crop species including corn and is not unique to biotechnology-enhanced crops. Pollen-mediated gene flow is a term used to describe the movement of plant genes from one plant to another via pollen. The rate of pollen-mediated gene flow depends on biotic and abiotic factors such as plant biology, pollen biology/volume, plant phenology, proximity of source and sink, ambient conditions such as temperature and humidity, and field architecture.

VIII.D.1. Vertical Gene Flow: Transfer of Genetic Information Within Cultivated *Zea* Species

Corn morphology fosters cross-pollination; therefore, high levels of pollen-mediated gene flow can occur in this species. Researchers recognize that 1) the amount of gene flow that occurs can be high because of open-pollination; 2) the percent gene flow will vary by population, hybrid or inbred; 3) the level of gene flow decreases with greater distance between the source and recipient plants; 4) environmental factors affect the level of gene flow; 5) corn pollen is viable for a short period of time under field conditions; 6) corn produces ample pollen over an extended period of time; and, 7) pollinating insects, especially bees, are occasional visitors to the tassels but rarely visit silks of corn.

There have been several studies conducted on the extent of pollen-mediated gene flow between corn fields. As expected, results were found to vary depending on the experimental design, environmental conditions and detection method. In general, the percent of gene flow was found to diminish with increasing distance from the source field, generally falling below 1% at distances >660 feet (Jemison and Vayda, 2000; Luna V. et al., 2001). The information is useful for managing gene flow during corn breeding, seed production, identity preservation or other applications, and forms the basis for the USDA-APHIS performance standards for corn. All testing and production of regulated MON 88017 seed or grain has been conducted under USDA notification according to these standards. Gene flow from fields planted with MON 88017 to other corn would not be of concern because of the lack of potential to cause harm to humans and to the environment.

VIII.D.1.a. Gene Flow to Wild Relatives

For gene flow to occur by normal sexual transmission, certain conditions must exist: 1) the two parents must be sexually compatible; 2) there must be overlapping phenology; and 3) a suitable pollen vector must be present and capable of transferring pollen between the two parents.

As discussed in Section II.G, corn and annual teosinte (*Zea mays* subsp. *mexicana* Schrad.) are genetically compatible, wind-pollinated and, in areas of Mexico and Guatemala, freely hybridize when in close proximity to each other. Corn easily crosses with teosinte; however, teosinte is not present in the U.S. other than as an occasional botanical garden specimen. These specimens would only flower at the same time as corn (because of photoperiod reaction) if they were subject to artificial day length shortening

for several weeks at a time (Wilkes, 1967). Differences in factors such as flowering time, geographical separation and development factors make natural crosses in the United States highly unlikely.

In contrast with corn and teosinte, which can be easily hybridized, both in the wild and by controlled pollinations, special techniques are required to hybridize corn and *Tripsacum*, and the offspring of the cross show varying levels of sterility (see Section II.G.). Only with extreme difficulty can corn be crossed with *Tripsacum* species. Furthermore, 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 to occur in the U.S.

VIII.D.1.b. Horizontal Gene Flow: Transfer of Genetic Information to Species with which Corn Cannot Interbreed

Monsanto is aware of no reports of the transfer of genetic material from corn to other species with which corn cannot sexually interbreed.

VIII.E. Current Agronomic Practices for U.S. Field Corn

This section provides a review of U.S. agronomic practices in corn and the anticipated environmental consequences from the commercialization of MON 88017. Included is a discussion of current corn production practices, weed occurrence and their management, rotational crops, and volunteer corn management.

VIII.E.1. Production

Corn is the largest U.S. crop in terms of acreage planted and net crop value. In 2003, approximately 79 million acres were planted to field corn in the U.S. (Table VIII-2). The acres planted to corn were similar in 2002, but approximately 4% lower in 2001. Seventy-one million acres or 90% of the total planted acres were harvested for grain in 2003. The remaining corn acres were harvested as silage for use as animal feed. In 2003, approximately 6.5 million acres or 8.2% of the total planted acres were harvested for silage.

In the U.S., total corn production was approximately 10.11 billion bushels (Bbu) in 2003, which was a record high. The previous record was set in 1994 with a total production of 10.10 Bbu (see Table VIII-2). Grain yield was also at a record high of 142.2 bu/A, which was up significantly from the yields of 138.2 and 130.0 bu/A recorded in 2001 and 2002, respectively. The value of the corn crop was $24.8 B in 2003, surpassing that of soybeans ($17.47 B) and wheat ($7.94 B) (USDA-NASS, 1995-2004). The value of corn production in the United States has ranged from $16.03 to $25.15 B in the past 11 years (Table VIII-2).

Field corn is planted in almost every state in the continental U.S. For convenience the states can be grouped into the following regions (number of states): Midwest (12), Northeast (8), Mid-Atlantic (5), Southeast (9), Great Plains (5), Northwest (4) and Southwest (5) (Table VIII-3). However, the majority of the corn (81%) is grown in the

Midwest region. Yields vary considerably from region to region because of rainfall/irrigation, climatic conditions and soil productivity. In 2003, average yields for the seven growing regions were as follows: 146 bu/A (Midwest), 117 bu/A (Northeast), 119 bu/A (Mid-Atlantic), 125 bu/A (Southeast), 122 bu/A (Great Plains), 168 bu/A (Northwest), and 166 bu/A (Southwest). The higher average yields obtained in the Northwest and Southwest regions are likely due to a greater percentage of corn grown under irrigation in these areas.

Table VIII-2. Field Corn Production in the U.S., 1993 – 2003[1]

Year	Acres Planted (x 1000)[1,2]	Yield (bu/A)[2]	Production (x 1000 bu)[2]	Value ($B)[3]
2003	79,066	142.2	10,113,887	24.80
2002	79,054	130.0	9,007,659	21.21
2001	75,752	138.2	9,506,840	18.89
2000	79,545	137.1	9,968,358	18.50
1999	77,431	133.8	9,437,337	17.10
1998	80,187	134.4	9,761,085	18.92
1997	80,227	127.0	9,365,574	22.35
1996	79,487	127.1	9,293,435	25.15
1995	71,245	113.5	7,373,876	24.12
1994	79,158	138.6	10,103,030	22.99
1993	73,239	100.7	6,336,470	16.03

[1] Data for 2003 from USDA-NASS, 2003a. [2]Data for 1993-2002 from USDA-NASS, 1995-2003. [3]USDA-NASS, 1995-2004.

Table VIII-3. Field Corn Production by Region and State in the U.S. in 2003

Region/State	Acres Planted[1] (x 1000)	Acres Harvested As Grain[2] (x 1000)	Average Yield[2] (bu/A)	Total Production[2] (bu)
Midwest region				
Illinois	11,100	11,050	164	1,812,200
Indiana	5,700	5,390	146	786,940
Iowa	12,400	12,000	157	1,884,000
Kentucky	1,230	1,080	137	147,960
Michigan	2,300	2,090	126	263,340
Minnesota	7,100	6,650	146	970,900
Missouri	2,950	2,800	108	302,400
Nebraska	8,000	7,700	146	1,124,200
North Dakota	1,450	1,170	112	131,040
Ohio	3,450	3,070	156	478,920
South Dakota	4,500	3,850	111	427,350
Wisconsin	3,700	2,850	129	367,650
Region Totals	**63,880**	**59,700**	**146[3]**	**8,696,900**

Table VIII-3 (Cont'd). Field Corn Production by Region and State in the U. S. in 2003

Region/State	Acres Planted[1] (x 1000)	Acres Harvested As Grain[2] (x 1000)	Average Yield[2] (bu/A)	Total Production[2] (bu)
Northeast Region				
New York	1,020	440	121	53,240
Pennsylvania	1,450	890	115	102,350
Connecticut[4]	30			
Maine[4]	26			
Massachusetts[4]	22			
New Hampshire[4]	16			
Rhode Island[4]	2			
Vermont[4]	96			
Region Totals	**2,662**	**1,330**	**117[3]**	**155,590**
Mid-Atlantic Region				
Delaware	180	162	123	19,926
Maryland	530	410	123	50,430
New Jersey	80	61	113	6,893
Virginia	480	330	115	37,950
West Virginia	45	27	115	3,105
Region Totals	**1,315**	**990**	**119[3]**	**118,304**
Southeast Region				
Alabama	230	190	122	23,180
Arkansas	350	350	140	49,000
Florida	85	39	82	3,198
Georgia	370	285	129	36,765
Louisiana	500	500	134	67,000
Mississippi	550	530	135	71,550
North Carolina	740	680	106	72,080
South Carolina	320	215	105	22,575
Tennessee	690	630	131	82,530
Region Totals	**3,835**	**3,419**	**125[3]**	**427,878**

Table VIII-3 (Cont'd). Field Corn Production by Region and State in the U. S. in 2003

Region/State	Acres Planted[1] (x 1000)	Acres Harvested As Grain[2] (x 1000)	Average Yield[2] (bu/A)	Total Production[2] (bu)
Great Plains Region				
Colorado	1,000	890	135	120,150
Kansas	2,900	2,500	120	300,000
Oklahoma	200	190	125	23,750
Texas	2,000	1,650	118	194,700
Wyoming	85	50	129	6,450
Region Totals	**6,185**	**5,280**	**122[3]**	**645,050**
Northwest Region				
Idaho	200	50	140	7,000
Montana	60	17	140	2,380
Oregon	65	30	170	5,100
Washington	130	70	195	13,650
Region Totals	**455**	**167**	**168[3]**	**28,130**
Southwest Region				
Arizona	45	22	190	4,180
California	500	170	160	27,200
Nevada[4]	4			
New Mexico	130	48	180	8,640
Utah	55	13	155	2,015
Region Totals	**734**	**253**	**166[3]**	**42,035**
US Totals	**79,066**	**71,139**	**142**	**10,113,887**

[1]USDA-NASS, 2003a.

[2] USDA-NASS, 2004.

[3]Average yield for the region = total production divided by total acres harvested.

[4] No production statistics reported by the USDA for these states.

VIII.E.2. Corn Cultivation and Management

The key considerations for corn production include soil quality, tillage practices, hybrid selection, moisture, nutrients, and the management of insects, weeds and diseases. A summary of the corn cultivation and management practices is provided below.

VIII.E.2.a. Soil Quality and Tillage Practices

Corn is grown in a variety of soils in the U.S. ranging from the sandhills of Nebraska and Colorado to the clays of delta regions, from strongly acidic to strongly alkaline soils, and from shallow soils on residual material to deep soils in loess, till or alluvium. Within these ranges, however, there is a corresponding variation in the crop's productivity. Ideal soils are those that have intermediate textures of loam to silt loam in the surface horizon and somewhat higher clay content as silt loam to silty clay loam in the subsoil. This combination, complemented with good structural properties, allows good storage of water and nutrients and a degree of permeability favorable to water intake and air exchange. Corn growth is optimal on soils with pH levels ranging between 6.0 and 7.0 and moderate-to-high fertility (Jones, 2003).

There are three main tillage practices employed in corn production: conventional tillage, reduced tillage, and conservation tillage. Conventional tillage practices leave <15% crop residue cover after planting and involve the use of a moldboard plow or other intensive tillage procedure. Reduced tillage practices leave between 15-30% crop residue cover after planting and exclude the use of a moldboard plow or other intensive tillage procedure. Conservation tillage is a system that covers 30% or more of the soil surface with crop residue after planting to reduce soil erosion by water, and consists of three subtypes: no-till, ridge-till and mulch-till. These subtypes differ in the timing of cultivation of the seedbed and type of equipment used. In recent years, there has been a trend toward the increased use of conservation tillage practices. In 1996, the total acres planted to corn were tilled as follows: conservation tillage (38%), reduced tillage (32%), and conventional tillage (30%) (USDA-ERS, 2002).

VIII.E.2.b. Corn Hybrids

Growers are confronted with virtually hundreds of corn hybrids marketed by seed companies. Their selection is primarily based on yield potential, climatic environment, and disease/pest resistance for that locale. One key determinant is the selection of seed with the appropriate maturity group for the local region (see Section II.E). Corn hybrids with relative maturities of 100 to 115 days are typically grown in the U.S. corn belt. To maximize yield, planting of corn on 30-in rows with a density of between 28,000 to 32,000 kernels per acre is typically recommended for the corn belt (Aldrich et al., 1986; Iowa State University, 2002).

VIII.E.2.c. Moisture and Nutritional Requirements

In the five major corn belt states, corn uses more moisture for evapotranspiration than is provided by rainfall, especially during the critical months of July and August (Shaw, 1988). The additional moisture requirement can be supplemented by irrigation, which is

practiced on 10-11 million acres or ~15% of the total corn acreage (USDA-ERS, 2002). Irrigation practices also facilitate the application of agricultural chemicals and fertilizers.

Corn, like all higher plants, requires at least 13 elements from the soil for growth and development (Olson and Sander, 1988). The 13 elements include the primary elements (nitrogen, phosphorus, and potassium), secondary elements (calcium, magnesium, sulphur), and micronutrient elements (iron, manganese, zinc, copper, boron, molybdenum and chlorine). By far the most important are the primary elements, which are depleted in the soil as the corn plant develops. Whereas nitrogen and phosphate uptake continues until maturity, potassium absorption is largely completed by the silking stage. This is why fertilization of corn fields is essential to ensure production and profitability. In 2002, nitrogen was applied on 96% of the corn acres at an average use rate of 137 lb/A per year. Phosphate and potassium fertilizers were applied on 79 and 68% of the corn acres, respectively (USDA-NASS, 2003b).

VIII.E.2.d. Management of Diseases and Insects

Management of diseases and insects during corn growth and development is essential for protecting the yield of the harvested grain. Estimates for annual yield losses because of diseases have ranged from 7 to 17% (Shurtleff, 1980). Incidence of disease infestation is highly variable and depends on many factors such as location, climate, and other environmental factors. Most corn hybrids on the market today have acceptable levels of resistance to common diseases. The major diseases found to occur in corn grown in the U.S. are summarized in Table VIII-4. In addition, several nematode species have been known to cause diseases in corn (Dicke and Guthrie, 1988). The use of fungicides in corn is limited because the incidence and severity of most diseases tends to be quite variable. In 2002, mancozeb and propiconazole were the two most common active ingredients used in the Midwest region for the control of diseases in corn (USDA-NASS, 2003b).

The corn crop is subject to attack by a complex of insects from the time it is planted until it is used as food and feed. The economically important insect pests in North America include wireworms, the black cutworm, European corn borer, Southwestern corn borer, the corn rootworm complex, grasshoppers, fall armyworm, and corn earworm. Table VIII-5 lists the major insect pests in corn grown in the U.S. Approximately 21 active ingredients are registered for use in corn for the control of insect pests. In its annual survey of agricultural chemical usage, USDA determined that 24 percent of the corn acreage was treated with insecticides in 2002 (USDA-NASS, 2003a).

The introduction of biotechnology-enhanced corn has offered growers an alternative and effective solution and for the control of major insect pests in corn. In 1996, Monsanto introduced YieldGard Corn Borer corn for the control of the European corn borer and other lepidopteran pests. The benefits of this technology, including insecticide reduction and environmental benefits, have been reviewed recently (James, 2003). In 2003, Monsanto introduced YieldGard Rootworm corn (MON 863) for the control of corn rootworm species. The benefits of this technology are discussed in Section I.B. The introduction of MON 88017 for the control of corn rootworm species will provide similar

benefits to growers combined with the ability to control weeds by the application of Roundup agricultural herbicides. The occurrence and management of weeds in corn is discussed in the following section.

VIII.E.3. Weeds in Corn

Weeds cause significant losses and require careful management by the grower because they interfere with corn plants by competing for available resources including water, nutrients and light. Economically damaging weeds in corn include annuals and perennials, grasses, broadleaf and sedge species. Some weeds can tolerate cold, wet conditions better than corn, and can get a head start prior to planting. Fields infested with perennial weeds present special problems for corn growers. Like annual weeds, perennials can reproduce by seeds, but they also regrow and spread vegetatively. This means that their rhizomes, thickened roots or tubers propogate new shoots, usually soon after corn is planted. Unless effectively controlled, perennial weeds can quickly gain a season-long advantage over the corn crop.

Corn yield loss is generally proportional to the amount of weeds present. While the ratio is not always one-to-one, some studies suggest that for every pound of weed dry matter, there is a reduction of approximately one pound of corn dry matter (grain, cobs, stalks and leaves) (Gianessi et al., 2002). Competition for light, nutrients, and moisture resources by the crop and weeds can lead to proportional reductions in yield (Knake et al., 1990). Numerous studies have shown that weed control early in the growing season is necessary to reduce yield losses in corn. Weed species such as giant foxtail, barnyardgrass and pigweed can reduce corn yields by up to 13, 35 and 50% respectively (Bosnic and Swanton, 1997; Fausay et al., 1997; Knake and Slife, 1965). In a study of mixed weed populations competing with corn, corn yields were reduced by up to 20% when the weed plants reached a height of eight inches (Carey and Kells, 1995).

Corn is typically planted in wide rows (30 inches) and has an upright leaf orientation. As a result, corn is not successful in competing with weeds early in the growing season. Corn is also usually planted early when soil temperature and weather conditions favor weed over corn growth. A survey of Extension Service weed scientists solicited estimates of the percent of corn acreage infested with individual weed species by state or region, as well as the the potential impact on corn yields if the species were left uncontrolled. In this survey, 12 annual broadleaf, nine annual grass, and seven perennial species were identified as troublesome weeds (Table VIII-6) (Gianessi et al., 2002). Estimates of yield loss ranged from a low of 15% due to wirestem muhly and sandburs to a high of 48% from burcucumber.

Pigweed is the most widespread weed species, infesting cornfields throughout the U.S. despite the fact that it is readily controlled by most corn herbicides. This is because it is a prolific seed producer and those seeds remain viable in the soil for years. Some weed species are problems at a regional level, e.g., hemp dogbane is a problem in Missouri and Illinois, wirestem muhly is a problem in Pennsylvania, and woolly cupgrass is a problem in Wisconsin and Iowa.

Table VIII-4. Major Diseases of Corn[1]

Common Name	Causative Agent [transmittal agent]
Seed rots and seedling blights	*Fusarium moniliform, Pythium* spp.
Foliar Diseases	
Bacterial leaf blight and stalk rot	*Pseudomonas avenae*
Bacterial stripe	*Pseudomonas andropogonis*
Stewart's wilt	*Erwinia stewartii*
Chocolate spot	*Pseudomonas coronafaciens*
Goss's wilt	*Clavibacter michiganense*
Holcus spot	*Pseudomonas syringe*
Anthracnose	*Colletotrichum graminicola*
Eyespot	*Kabatiella zeae*
Gray leaf spot	*C. zeae-maydis*
Northern leaf spot	*Bipolaris zeicola*
Northern corn leaf blight	*Exserohilum turcicum*
Physoderma brown spot	*Physoderma maydis*
Southern corn leaf blight	*Bipolaris maydis*
Yellow leaf blight	*Phyllosticta maydis*
Common rust	*Puccinia sorghi*
Southern corn rust	*Puccinia polysora*
Common corn smut	*Ustilago maydis*
Systemic Diseases	
Head smut	*Sphacelotheca reiliana*
Crazy top	*Sclerophthora macrospora*
Sorghum downy mildew	*Peronosclerospora sorghi*
Maize dwarf mosaic virus	[aphids]
Maize chlorotic dwarf virus	[leafhoppers]
Corn lethal necrosis	[chrysomelid beetles]
Maize white line mosaic virus	[not identified]
Corn stunt	[leafhoppers]
Maize bushy stunt	[leafhoppers]
Stalk and root rots	
Gibberella stalk rot	*Giberrella zeae*
Diplodia stalk rot	*Stenocarpella maydis*
Anthracnose stalk rot	*Colletotrichum graminicola*
Charcoal rot	*Macrophomina phaseolina*
Fusarium stalk rot	*Fusarium moniliforme*
Pythium stalk rot	*Pythium aphanidermatum*
Bacterial stalk rot diseases	*Erwinia chrysanthemi*
Root rots	*Pythium* spp.
Ear rots and storage molds	
Fusarium ear rot	*Fusarium moniliforme*
Giberrela ear rot	*Gibberella zeae*
Diplodia ear rot	*Diplodia maydis*
Aspergillus ear and kernel rot	*Aspergillus flavus*
Storage molds	*Penicillium* spp., *Aspergillus* spp.

[1]Smith and White, 1988.

Table VIII-5. Major Insect Pests of Corn[1]

Common Name	Latin name
Soil Insects	
Northern corn rootworm	*Diabrotica barberi*
Western corn rootworm	*Diabrotica virgifera virgifera*
Southern corn rootworm	*Diabrotica undecimpunctata*
Black cutworm	*Agrotis ipsilon*
Wireworms	*A. mancus, Horistonotus uhlerii, Melanotus cribulosus,* others
Billbugs	*Sphenophorus* spp.
White grubs	*Phyllophaga* spp.
Corn root aphid	*Anuraphis maidiradicis*
Seedcorn maggot	*Delia platura*
Grape colaspis	*Colaspis brunnea*
Seed corn beetle	*Stenolophus lecontei*
Insects attacking the leaf, stalk, and ear	
Corn earworm	*Heliothis zea*
European corn borer	*Ostrinia nubilalis*
Corn leaf aphid	*Rhopalosiphum maidis*
Fall armyworm	*Spodoptera frugiperda*
Stalk borers	*Diatraea spp.*
Armyworm	*Pseudaletia unipuncta*
Lesser stalk borer	*Elasmopalpus lignosellus*
Chinch bug	*Blissus leucopterus leucopterus*
Grasshoppers	*Melanoplus differentialis*
Corn flea beetle	*Chaetocnema pulicaria*
Japanese beetle	*Popillia japonica*
Other insects	
Thrips	*Anaphothrips* spp., *Frankliniella* spp.
Leafhoppers	*Trigonotylus brevipes,* others
Western bean cutworm	*Loxagrotis albicosta*
Corn blotch leaf miner	*Agromyza parvicornis*
Spider mites	*Oligonychus* spp., *Tetranychus* spp.
Pink scavenger caterpillar	*Pyroderces rileyi*
Garden symphlan	*Scuttigerella immaculata*
Hop-vine borer	*Hydraecia immanis*
Sod webworms	Subfamily Cramdinae
Leaf rollers	
Stink bugs	
Insect disease vectors	Several

[1] Dicke and Guthrie, 1988 and University of Missouri, 1998.

Table VIII-6. Troublesome Weeds in U.S. Corn[1]

Weed Species	Area Infested[2]	Acreage Infested (%)	Potential Yield Loss (%)
Annuals			
Broadleaves			
Burcucumber	PA/OH/TN/SE	5-10	48
Cocklebur	MW/NP/SE	20-60	33
Jimsonweed	MW/CO	5-20	17
Kochia	NP/NW	10-70	33
Lambsquarters	MW/SE/NE/CA	15-80	33
Morningglory	MW/SE/SP	20-75	33
Nightshade	MW/NP/CA	25-50	26
Pigweeds/Waterhemp	US	30-90	36
Ragweed, Common	MW/SE/NE	20-70	30
Ragweed, Giant	MW/NP	10-45	28
Smartweeds	MW/SD/NE/SE	30-70	22
Velvetleaf	MW/NE/NP	25-70	28
Grasses			
Barnyardgrass	SP/NW/CA	80-90	23
Bermudagrass	MD/SE/UT/CA	10-20	47
Crabgrass spp.	MW/SE/NE	20-80	29
Cupgrass, Woolly	IA/WI	15-20	29
Foxtail spp.	MW/NE/NP	50-90	31
Millet, Wild-Proso	UT/WY/CO/ID	15-40	31
Panicum, Fall	MW/SE/NE/NP	15-80	30
Sandburs	NP/UT/WY	5-30	15
Shattercane	MW/SP	5-40	33
Perennials			
Bindweed, Field	ND/SW/CA	40-80	18
Dogbane, Hemp	IL/MO	2-20	21
Johnsongrass	MW/SE/SW/CA	20-60	45
Muhly, Wirestem	PA	2	15
Nutsedge, Yellow	MW/SE/NE/NP/CA	10-70	21
Quackgrass	MW/NE/UT	10-70	27
Thistle, Canada	NE/MW/NP/CO	5-25	26

[1]Gianessi et al., 2002.

[2]***Regions***

MW: Midwest
NE: Northeast
NP: Northern Plains
NW: Northwest
SE: Southeast
SW: Southwest
SP: Southern Plains
US: United States

States

CA: California
CO: Colorado
ID: Idaho
IA: Iowa
MD: Maryland
MO: Missouri
ND: North Dakota
OH: Ohio
PA: Pennsylvania
SD: South Dakota
TN: Tennessee
UT: Utah
WI: Wisconsin
WY: Wyoming

The regions match those described in Section VIII.E. The Northern (CO, KS, WY) and Southern (OK, TX) plains are subdivisions of the Great Plains region.

VIII.E.4. Weed Management in Corn

Until the early 1950s, tillage and cultivation practices were primarily used for weed control in corn, but since then they have been largely replaced by the use of herbicides. This transition was accelerated by the introduction of 2,4-D, which became the first commonly used herbicide in corn in the 1950s. It was used as a postemergence herbicide to kill a broad spectrum of annual and some perennial broadleaf weeds. In the 1960s, growers began to use soil-applied preplant incorporated herbicides such as EPTC and butylate to control several grass weeds including foxtails that had become a problem as a result of the control of broadleaf weeds with the use of 2,4-D (Pike et al., 1991). Atrazine was introduced in 1959 and became a widely used soil-applied herbicide due its ability to control a broad spectrum of weeds, including several grass and troublesome broadleaf species, with excellent crop tolerance. Consequently, soil applications of atrazine largely replaced 2,4-D.

Herbicide use in corn became widespread by the end of the 1970s. Surveys indicate that the percentage of corn acres treated with herbicides reached 79% of the acreage in 1971 and this has remained over 95% since 1982 (Andrilenas, 1974; USDA-ERS, 1983). Combinations of atrazine with herbicides such as alachlor and metolachlor, which extended the grass control spectrum, became common in the 1970s and 1980s (Gianessi et al., 2002). Atrazine exhibits both residual soil weed control activity as well as contact postemergence control properties. The use of postemergence treatments that included atrazine in combination with other herbicides, such as bromoxynil and bentazon, increased in the 1980s (Pike et al., 1991). The use of postemergence herbicide treatments increased in corn in the late 1980s and early 1990s because of the introduction of new herbicides, notably nicosulfuron and primisulfuron, which significantly improved selective control of grasses. These herbicides provided more effective control of certain annual grass species such as shattercane and certain perennial grasses such as johnsongrass. With the introduction of Roundup Ready corn in 1998, the use of over-the-top applications of Roundup agricultural herbicides for broadspectrum weed control became available to the grower.

Postemergence treatments have increased in corn in part because of the variable performance of soil-applied herbicides based on soil type and amount and timing of rainfall. Soil-applied herbicides are dependent on rainfall or irrigation for activation and movement into the soil. At some locations, late-season control declines with preemergence treatments.

Table VIII-7 shows estimates of the herbicide active ingredients used in corn in the U.S. in 2003 (AgroTrak® annual surveys; Doane, 2003). Forty-eight herbicide active ingredients were used for weed control in corn, representing 12 different classes and three subclasses of modes of action (Table VIII-8). Atrazine was the most extensively used herbicide active ingredient for weed control in corn with applications on 67.2% of the corn acres. Acetochlor and s-metolachlor were applied on 23.7% and 24.3% of the corn

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acres, respectively. Atrazine was frequently applied in tank mixtures with acetochlor and s-metolachlor to broaden the spectrum of control. Dicamba, glyphosate, mesotrione, nicosulfuron, and rimsulfuron were each applied on greater than 10% of the corn acres. The primary postemergence herbicide active ingredients were dicamba, glyphosate, 2,4-D, nicosulfuron and primisulfuron. Postemergence herbicide applications to corn often include low rates of atrazine in a mixture or prepack for improved control and broader spectrum.

Table VIII-7. Survey of Herbicide Active Ingredient Usage in the U.S. in 2003[1]

Active Ingredient	Herbicide HRAC[2] Classification	% of Planted Acres	Total Applied (lb ai)
Atrazine	C1	67.25	66,174,119
Glyphosate	G	24.82	17,306,048
Metolachlor-s	K3	24.26	25,165,132
Acetochlor	K3	23.70	33,579,613
Dicamba	O	16.97	2,658,087
Nicosulfuron	B	12.58	203,884
Mesotrione	F2	10.78	870,860
Rimsulfuron	B	10.68	97,531
2,4-D	O	8.44	3,371,492
Isoxaflutole	F2	8.28	366,550
Flumetsulam	B	7.31	202,839
Clopyralid	O	6.86	560,477
Dimethenamid	K3	4.77	3,580,653
Diflufenzopyr-Na	P	4.34	151,188
Simazine	C1	3.93	3,614,975
Primisulfuron-methyl	B	3.24	56,714
Glufosinate-ammonium	H	2.99	821,299
Pendimethalin	K1	2.98	2,315,007
Imazethapyr	B	2.57	85,368
Imazapyr	B	2.41	26,106
Dimethenamid-p	K3	2.26	1,413,315
Alachlor	K3	2.04	3,064,658
Flufenacet	K3	1.98	649,851
Paraquat	D	1.83	752,758
Prosulfuron	B	1.52	11,657
Bromoxynil	C3	1.42	296,321
Carfentrazone-ethyl	E	1.30	7,225
Foramsulfuron	B	1.21	23,900
Halosulfuron-methyl	B	0.65	15,621
Thifensulfuron-methyl	B	0.58	3,342
Metribuzin	C1	0.51	41,525
Sulfosate	G	0.22	177,347

Table VIII-7 (cont'd). Survey of Herbicide Active Ingredient Usage in the U.S. in 2003[1]

Active Ingredient	Herbicide HRAC[2] Classification	% of Planted Acres	Total Applied (lb ai)
Ametryn	C1	0.17	153,951
EPTC	N	0.15	421,154
Iodosulfuron	B	0.11	153
Cyanazine	C1	0.09	35,282
Trifluralin	K1	0.09	50,664
Pelargonic acid	Z	0.07	54,145
Butylate	N	0.06	175,175
Flumiclorac-pentyl	E	0.06	626
Bentazon	C3	0.05	25,229
Flumioxazin	E	0.05	2,532
Linuron	C2	0.04	42,817
Metolachlor	K3	0.04	63,264
Diuron	C2	0.03	14,143
Sethoxydim	A	0.02	677
Tribenuron-methyl	B	0.02	76
Oxyfluorfen	E	0.001	504

[1]Data from the Agrotrak survey (Doane, 2003). [2]HRAC = Herbicide Resistance Action Committee (HRAC, 2003). See Table VIII-8.

Table VIII-8. The HRAC Classification System for Herbicides[1]

HRAC Group	Mode of Action	Example Herbicide
A	Inhibition of acetyl CoA carboxylase (ACCase)	Diclofop-methyl
B	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS)	Chlorsulfuron
C1	Inhibition of photosynthesis at photosystem II	Atrazine
C2	Inhibition of photosynthesis at photosystem II	Chlorotoluron
C3	Inhibition of photosynthesis at photosystem II	Bromoxynil
D	Photosystem-I-electron diversion	Paraquat
E	Inhibition of protoporphyrinogen oxidase (PPO)	Oxyfluorfen
F1	Bleaching: Inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS)	Flurtamone
F2	Bleaching: Inhibition of 4-hydroxyphenyl-pyruvate dioxygenase (4-HPPD)	Mesotrione
F3	Bleaching: Inhibition of carotenoid biosynthesis (unknown target)	Amitrole
G	Inhibition of EPSP synthase	Glyphosate
H	Inhibition of glutamine synthetase	Glufosinate ammonium
I	Inhibition of DHP (dihydropteroate synthase)	Asulam
K1	Microtubule assembly inhibition	Trifluralin
K2	Inhibition of mitosis / microtubule organization	Propham
K3	Inhibition of cell division (Inhibition of very long chain fatty acids)	Butachlor
L	Inhibition of cell wall (cellulose) synthesis	Dichlobenil
M	Uncoupling (membrane disruption)	Dinoseb
N	Inhibition of lipid synthesis - not ACCase inhibition	Triallate
O	Synthetic auxins (action like indole acetic acid)	2,4-D
P	Inhibition of auxin transport	Naptalam
Z	Unknown	Flamprop-methyl

[1]HRAC, 2003.

Mixtures of herbicide active ingredients used with conventional corn cultivars provide fair to excellent control of most of the troublesome weed species in corn. Notable exceptions are bermudagrass, field bindweed, hemp dogbane, and wirestem muhly. A species that is becoming more problematic in cornfields is shattercane because of the development of populations resistant to applications of ALS herbicides such as primisulfuron, nicosulfuron and rimsulfuron.

VIII.E.5. Efficacy of Glyphosate-based and Other Corn Herbicides

Preemergence and postemergence herbicides vary greatly in their effectiveness in controlling weed species that occur in corn fields. Selective herbicides are generally more effective on either the grass or broadleaf spectrum of weed species. Therefore, herbicides are frequently tank-mixed or premixed into a single product to achieve a broader spectrum of weed control. Tables VIII-9 and VIII-10 provide weed control ratings for most of the individual and premix herbicides on many of the common annual and perennial weeds in corn (Ohio State University, 2004). These tables compare the relative effectiveness of herbicides on individual weeds. Ratings are based on labeled application rate and weed size or growth stage. Performance may be better or worse than indicated in the tables, because of weather or soil conditions or other variables. Listed below are an explanation of the ratings and abbreviations used in the Tables VIII-9 and VIII-10.

Weed control rating:	Crop tolerance rating:
9 = 90 to 100% control	0 = Excellent
8 = 80 to 90% control	1 = Good
7 = 70 to 80% control	2 = Fair
6 = 60 to 70% control	3 = Poor
- = insufficient data	Crop injury of 1 or less is rarely significant
A weed control rating of 5 or less is rarely significant	

Products listed in Tables VIII-9 and VIII-10 containing premixtures of two or more active ingredients are described in Table VIII-11. The boldface numbers represent weed control ratings of 8 or higher. None of the individual or premix products are rated 8 or higher on all the weed species common in corn. Growers must know the specific weed problems present on their farm or fields to select the most effective herbicide program. This decision is much easier with Roundup Ready corn because of the broad weed control spectrum of glyphosate.

Glyphosate is rated 8 or higher on all the weed species except yellow nutsedge and annual morningglory, which were rated at 7 and 6, respectively (Table VIII-10). None of the other herbicides offers such effective broadspectrum control in corn. Many of the other postemergence herbicides in corn provide control of either the grass or broadleaf spectrum of weeds only. Some of the postemergence products contain up to three active ingredients and still do not provide the spectrum or level of control provided by glyphosate. Another important factor for growers to consider in herbicide selection is

crop tolerance. Several of the herbicides listed can provide control of several grass and broadleaf weeds, but are rated only fair for crop safety. Although glyphosate is a nonselective herbicide providing control grass and broadleaf species of weeds, it is rated excellent for crop tolerance when applied to Roundup Ready corn. In addition, glyphosate has good efficacy against hard to control perennial species such as Canada thistle, yellow nutsedge, quackgrass and rhizome johnsongrass. Glyphosate provides consistent control across a wide variety of weather and soil conditions that are also important factors in selecting a herbicide program.

VIII.F. Impact of the Introduction of MON 88017 on Agronomic Practices

VIII.F.1. Impact on Cultivation and Management Practices

No negative impact is expected from the introduction of MON 88017 on current cultivation and management practices for corn as described in Section VIII.E.2. MON 88017 has been shown to be no different than conventional corn in its phenotypic, ecological, and compositional characteristics (Section VII). Thus, MON 88017 is expected to be similar in its agronomic characteristics, and have the same levels of resistance to insects and diseases as current commercial corn, except for enhanced protection from feeding damage caused by corn rootworm insects. The use of MON 88017 combined with over-the-top applications of Roundup agricultural herbicides likely will have a beneficial impact on the adoption of conservation tillage practices, previously noted for Roundup Ready corn (see discussion below).

The impact of the introduction of MON 88017 on weed and insect control and on crop rotational practices in corn is discussed in the following sections.

VIII.F.2. Impact on Weed Control Practices

The use of MON 88017 in combination with over-the-top applications of Roundup agricultural herbicides will offer growers the same weed control benefits as commercial Roundup Ready corn. Based on the label for Roundup WeatherMAX herbicide, growers can make sequential applications from emergence through the V8 stage, or until a corn plant height of 30 inches, whichever comes first. When corn plant height is >30 inches, later-emerging weeds can be controlled by the use of drop nozzle applications. Provided labeled directions are followed, Roundup WeatherMAX herbicide, or any other Roundup agricultural herbicide, will provide control or suppression of most annual and perennial species listed in Tables VIII-9 and VIII-10.

Table VIII-9. Efficacy of Preplant and Preemergence Herbicides in Corn[1]

			Grasses									Broadleaf Weeds														
Product	**HRAC Group**	**Crop Tolerance**	**Barnyardgrass**	**Crabgrass**	**Fall Panicum**	**Giant Foxtail**	**Yellow Foxtail**	**Shattercane**	**Seedling Johnsongrass**	**Rhizone Johnsongrass**	**Quackgrass**	**Yelllow Nutsedge**	**Annual Morningglory**	**Black Nightshade**	**Burcucumber**	**Cocklebur**	**Common Ragweed**	**ALS-Resistant Common Ragweed**	**Giant Ragweed**	**ALS-Resistant Giant Ragweed**	**Jimsonweed**	**Lambsquarters**	**Triazine-Resistant Lambsquarters**	**Pigweed (redroot)**	**Smartweed**	**Velvetleaf**
Preplant or Preemergence																										
Acetochlor	K3	1	**9**	**9**	**8**	**9**	**9**	5	4	0	0	**8+**	0	**8+**	0	0	7	7	2	2	4	7+	7+	**8+**	5	3
Acetochlor + Atrazine	K3/C1	1	**9**	**9**	**8**	**9**	**9**	5	4	0	6	**8+**	**8**	**9**	6	**8**	**9**	**9**	**8**	**8**	**9**	**9**	7+	**9**	**9**	**8**
Atrazine	C1	0	**8**	5	3	7	7	2	0	0	**8**	7	**8**	**9**	6	**8**	**9**	**9**	**8**	**8**	**9**	**9**	0	**9**	**9**	**8**
Axiom®	K3/C1	1	**8**	**8+**	**8**	**8+**	**8+**	5	4	0	0	6	3	**8**	0	2	7	7	2	2	6	**8**	5	**9**	7	7
Balance® Pro	F2	2	**8**	7	**8**	**8**	6	5	2	0	0	3	5	**9**	7	3	**9**	**9**	6	6	**9**	**9**	**9**	**9**	**8**	**9**
BicepII Magnum®	K3/C1	1	**9**	**9**	**8**	**9**	**9**	5	4	0	6	**8**	**8**	**9**	6	**8**	**9**	**9**	**8**	**8**	**9**	**9**	6	**9**	**9**	**8**
Callisto™	F2	1	3	6	3	3	3	0	0	0	0	0	6	**9**	7	5	7	7	6	6	-	**9**	**9**	**9**	**9**	**9**
Define™	K3	1	**8**	**8+**	**8+**	**8+**	**8+**	5	4	0	0	6	0	**8**	0	0	5	5	2	2	4	6	6	**8**	4	2
Dual II Magnum®	K3	1	**8**	**9**	**8+**	**9**	**9**	5	4	0	0	**8+**	0	**8**	0	0	5	5	2	2	4	6	6	**8**	4	2
Epic®	K3/F2	2	**8**	**9**	**9**	**9**	**9**	5	5	0	0	6	5	**9**	7	3	**9**	**9**	6	6	**8+**	**9**	**9**	**9**	**8**	**9**
Guardsman Max™	K3/C1	1	**9**	**8+**	**8**	**8+**	**8+**	5	4	0	5	**8**	**8**	**9**	5	**8**	**9**	**9**	7	7	**9**	**9**	6	**9**	**9**	7+
Hornet®	B/O	2	0	0	0	0	0	0	0	0	0	0	6	**8+**	3	**8**	**8+**	**8+**	7+	7+	**8**	**9**	**9**	**9**	**8+**	**9**
Lariat®/Bullet®	K3/C1	1	**9**	**8+**	**8**	**8+**	**8+**	5	4	0	6	**8**	**8**	**9**	6	**8**	**9**	**9**	**8**	**8**	**9**	**9**	6	**9**	**9**	**8**
Lumax™	F2/K3/C1	1	**9**	**9**	**8**	**9**	**9**	5	4	0	6	**8**	**8**	**9**	7	**8**	**9**	**9**	**8**	**8**	**9**	**9**	**9**	**9**	**9**	**9**
Lasso®/MicroTech®	K3	1	**8**	**8+**	**8**	**8+**	**8+**	5	4	0	0	**8**	0	**8+**	0	0	5	5	2	2	4	6	6	**8**	4	0
Outlook™	K3	1	**8**	**8+**	**8**	**8+**	**8+**	5	4	0	0	**8**	0	**8+**	0	0	5	5	2	2	4	6	6	**8**	4	2
Python™	B	2	0	0	0	0	0	0	0	0	0	0	5	**8**	3	7	7	0	5	0	7	**9**	**9**	**9**	**8**	**8+**
Simazine	C1	0	**8**	7	7	**8**	**8**	4	2	0	6	2	7	**9**	6	7	**9**	**9**	7	7	**8**	**9**	0	**9**	**8+**	7
Preemergence																										
Lorox®/Linex®	C2	2	5	5	5	5	5	0	0	0	0	0	2	7	0	6	**8**	**8**	5	5	6	**9**	**9**	**9**	**9**	6
Prowl®/Pendimax™	K1	2	**8**	**8**	**8**	**8**	**8**	6	6	2	0	0	0	0	0	0	2	2	0	0	2	**8**	**8**	**9**	3	4

[1]Acetochlor and acetochlor atrazine are available from a number of manufacturers. Grass weed control ratings presented here are for Degree products, which can provide a longer period of grass control compared to other acetochlor products. Broadleaf weed control ratings assume an atrazine rate of 1.5 lbs ai/A – atrazine rate in some acetochlor/atrazine premix products may be lower. Boldface numbers represent weed control ratings of 80-100% (see text).

Table VIII-10. Efficacy of Postemergence Herbicides in Corn

			Grasses									Broadleaf Weeds															
Product	**HRAC Group**	**Crop Toelrance**	**Barnyardgrss**	**Crabgrass**	**Fall Panicum**	**Giant Foxtail**	**Yellow Foxtail**	**Shattercane**	**Seedling Johnsongrass**	**R.hizone Johnsongrass**	**Quackgrass**	**Yellow Nutsedge**	**Annual Morningglory**	**Black Nightshade**	**Burcucumber**	**Cocklebur**	**Common Ragweed**	**ALS-Resistant Common Ragweed**	**Giant Ragweed**	**ALS-Resistant Giant Ragweed**	**Jimsonweed**	**Lambsquarters**	**Triazine-Resistant Lambsquarters**	**Pigweed (redroot)**	**Smartweed**	**Velvetlef**	**Canada Thisle**
Postemergence																											
2, 4-D	O	2	0	0	0	0	0	0	0	0	0	0	**9**	7	3	**9**	**9**	**9**	**9**	**9**	7	**9**	**9**	**9**	6	**8**	6
Accent®	B	1	**8+**	4	**8+**	**9**	**9**	**9**	**9**	**9**	**9**	6	**8**	0	**8**	5	0	0	0	0	**8**	5	5	**9**	**8**	5	6
Aim™	E	2	0	0	0	0	0	0	0	0	0	0	**8**	**8**	3	3	6	6	3	3	3	7	7	**8+**	5	**9**	2
Atrazine	C1	1	7	5	5	**8**	**8**	2	0	0	7	7	**9**	**9**	**8**	**9**	**9**	**9**	**8**	**8**	**9**	**9**	0	**9**	**9**	**8**	6
Basagran®	C3	0	3	0	0	0	0	0	0	0	0	8	4	3	2	**9**	7	7	6	6	**9**	6	6	4	**9**	**8+**	7
Basis®	B	2	7	6	7	7	7	7	7	4	-	-	6	0	-	7	6	0	0	0	4	**8**	**8**	**9**	**8**	**9**	5
Basis Gold®	B/C1	2	**8+**	4	**8**	**9**	**8**	**9**	**9**	6	7	6	**8+**	7	**8**	7	**9**	**8**	7	7	**8**	**9**	5	**9**	**9**	7	7
Beacon®	B	2	2	4	**8**	7	7	**9**	**9**	7	**8+**	6	6	**8**	**9**	**9**	**9**	0	**9**	0	**9**	5	5	**9**	**8**	**8**	6
Bromoxynil	C3	1	0	0	0	0	0	0	0	0	0	0	**8**	**9**	7	**9**	**9**	**9**	**8**	**8**	**9**	**9**	**9**	7	**8**	**8**	6
Bormoxynil + Atrazine	C3/C1	1	2	2	0	2	2	0	0	0	0	2	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	7+
Callisto™	F2	1	0	7*	0	0	0	0	0	0	0	5	7	**9**	-	7	**8**	**8**	**9**	**9**	-	**9**	**9**	**8**	**9**	**9**	5
Celebrity® Plus	B/O	2	**8+**	4	**8+**	**9**	**9**	**9**	**9**	**9**	**9**	7	**9**	**8**	**8+**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**8**	**9**
Dicamba	O	2	0	0	0	0	0	0	0	0	0	0	**9**	**8**	7	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**8**	7+	7
Dicamba + Atrazine	O/C1	2	2	2	0	2	2	0	0	0	0	2	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	7+
Distinct®	O	2	6	6	6	6	6	0	0	0	0	0	**9**	**8**	7	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**8+**	**8**	**9**
Equip™	B	1	**8+**	7	7+	**9**	7	**9**	**9**	**8+**	**8**	3	6	**9**	**8**	**8**	**8**	0	**8**	0	**8+**	**8**	**8**	**9**	**8**	**8+**	6
Exceed®	B	1	0	0	7	5	5	**9**	**9**	5	5	5	7+	**8**	**9**	**9**	**9**	0	**9**	0	**9**	**8**	**8**	**9**	**9**	**9**	6
Glyphosate[1]	G	0	**8**	**8**	**8**	**9**	**9**	**9**	**9**	**9**	**9**	7	6	**8**	**8**	**9**	**8+**	**8+**	**8+**	**8+**	**9**	**8+**	**8+**	**9**	**8**	**8**	**9**
Hornet®	B/O	1	0	0	0	0	0	0	0	0	0	0	7	7	6	**9**	**9**	**9**	**9**	**9**	7	7+	7+	7+	**9**	**8+**	**8+**
Laddok®	C1/C3	1	2	2	0	2	2	0	0	0	0	**8+**	**8**	**8**	6	**9**	**9**	**9**	**9**	**9**	**9**	**9**	5	**9**	**9**	**9**	7+
Liberty®[2]	H	0	7	**8**	**8**	**9**	7	**8**	**8**	7	6	5	**8**	**9**	**8**	**9**	**9**	**9**	**9**	**9**	**9**	**8**	**8**	**8**	**9**	**8**	6
Liberty® ATZ[2]	H/C1	0	7	7	7	**9**	7	7	7	7	7	6	**9**	**9**	**8**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**8**	**9**	**9**	**9**	7+
Lightning®[3]	B/B	1	7	7	7	**8**	**8**	**8+**	**8+**	7	3	5	7+	**9**	6	**9**	7	0	**8**	0	**8+**	**8+**	**8+**	**9**	**9**	**9**	6
Northstar™	B/O	2	2	4	7	6	6	**9**	**9**	6	7	5	**8**	**9**	**9**	**9**	**9**	7	**9**	6	**9**	**9**	**9**	**9**	**9**	**8+**	7
Option®	B	1	**8+**	7	**8**	**9**	7	**9**	**9**	**8+**	**8+**	3	5	**9**	**8**	7	7	0	5	0	**8**	7	7	**8**	6	**8**	5
Permit®	B	1	0	0	0	0	0	0	0	0	0	**9**	6	4	5	**9**	**8**	0	**8**	0	**8**	5	5	**9**	7	**8**	6
Priority™	B/E	2	0	0	0	0	0	0	0	0	0	**9**	**8**	**8**	5	**9**	**8**	6	**8**	3	**8**	7	7	**9**	**8**	**9**	6
Resource®	E	2	0	0	0	0	0	0	0	0	0	0	4	4	4	7	7	7	4	4	7	7	7	**9**	4	**9**	3
Shotgun®	C1/0	2	5	3	3	6	6	0	0	0	4	5	**9**	**9**	7	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**8+**	3
Spirit™	B	2	2	2	7	6	6	**9**	**9**	6	7	5	7	**8**	**9**	**9**	**9**	0	**9**	0	**9**	6	6	**9**	**8+**	**8+**	6
Steadfast®	B	2	**8**	4	**8**	**9**	**9**	**9**	**9**	**8**	**8**	5	6	0	7	6	4	0	3	0	6	4	4	**9**	7	5	6
Steadfast® ATZ	B/C1	2	**8**	7	**8**	**9**	**9**	**9**	**9**	**8**	**8**	7	**8+**	7	**8**	**9**	**9**	**8**	**8**	7	**9**	**9**	4	**9**	**9**	**8**	6
Stinger™	O	0	0	0	0	0	0	0	0	0	0	0	0	**9**	2	**9**	**9**	**9**	**9**	**9**	**8**	0	0	0	5	0	**9**
Yukon™	B/O	2	0	0	0	0	0	0	0	0	0	**9**	**8**	7	7	**9**	**9**	7	**9**	7	**9**	**8**	**8**	**9**	**9**	**9**	7

[1]Apply to Roundup® Ready (glyphosate-tolerant) corn only.
[2]Apply to Liberty Link (glufosinate-resistant) corn only.
[3]Apply to Clearfield (imidazolinone-resistant or tolerant) corn only.
*Large crabgrass only. Boldface numbers represent weed control ratings of 80-100% (see text).

Table VIII-11. Herbicide Products and Active Ingredients

Herbicide	Active Ingredient	Herbicide	Active Ingredient
Accent®	nicosulfuron	Lariat®/Bullet®	alachlor + atrazine
Aim™	carfentrazone	Lasso®/Micro-Tech®	alachlor
Axiom®	flufenacet + metribuzin	Liberty®	glufosinate
Balance® Pro	isoxaflutole	Liberty® ATZ	glufosinate + atrazine
Basagran®	bentazon	Lightning®	imazethapyr + imazapyr
Basis®	rimsulfuron + thifensulfuron	Lorox®/Linex®	linuron
Basis Gold®	rimsulfuron + nicosulfuron + atrazine	Lumax™	mesotrione + s-metolachlor + atrazine
Beacon®	primisulfuron	Northstar™	primisulfuron + dicamba
BicepII Magnum®	s-metolachlor + atrazine	Option®	foramsulfuron
Callisto™	mesotrione	Outlook™	dimethenamid
Celebrity® Plus	diflufenzopyr + dicamba + nicosulfuron	Permit®	halosulfuron
Define™	flufenacet	Priority™	carfentrazone + halosulfuron
Degree™	acetochlor	Prowl®/Pendimax™	pendimethalin
Distinct®	diflufenzopyr + dicamba	Python™	flumetsulam
Dual II Magnum®	s-metolachlor	Resource®	flumiclorac pentyl ester
Epic®	flufenacet + isoxaflutole	Shotgun®	atrazine + 2,4-D
Equip™	foramsulfuron + iodosulfuron	Spirit™	prosulfuron + primisulfuron
Exceed®	prosulfuron + primisulfuron	Steadfast® ATZ	nicosulfuron + rimsulfuron + atrazine
Guardsman Max™	dimethenamid + atrazine	Steadfast®	nicosulfuron + rimsulfuron
Hornet®	clopyralid + flumetsulam	Stinger™	clopyralid
Laddock®	bentazon + atrazine	Yukon™	halosulfuron + dicamba

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Effective weed management in MON 88017 corn will require using the correct rate of glyphosate based on size for the weed species present. Some agronomic conditions will also favor the use of other herbicides or weed control practices. As shown in Table VIII-7, 48 different herbicide active ingredients from a variety of classes can be used to control problem weeds in corn. The most commonly used herbicide active ingredients (>10% of the corn acres) are atrazine, a photosynthesis II inhibitor (class C1); acetochlor and s-metolachlor, cell division inhibitors (class K3); dicamba, a growth regulator (class O); glyphosate, a EPSP synthase inhibitor (class G); mesotrione, a pigment inhibitor (class F1); and nicosulfuron and rimsulfuron, inhibitors of ALS (class B).

Recommendations for weed control in MON 88017 corn will include the use of premixes and other herbicides with glyphosate as appropriate based on weed spectrum and other agronomic purposes. The following provides a summary of these and other benefits of the Roundup Ready corn system.

Effective broadspectrum weed control. The Roundup Ready corn system provides excellent control of most annual grass and broadleaf species. Most corn herbicides, particularly postemergence herbicides control a limited spectrum of weeds. In addition, Roundup agricultural herbicides provide excellent weed control over a wide range of climatic and soil conditions.

Control of perennial weed species. Roundup agricultural herbicides provide effective in-crop control of many problem perennial weeds in corn, namely quackgrass, johnsongrass and wirestem muhly.

Wide window of herbicide application. Growers desire and need more flexibility on herbicide application timing while still maintaing effective weed control and excellent crop safety. In less than ideal weather conditions in the spring, a wide window of application offers greater flexibility to schedule applications. Roundup agricultural herbicides will control problem weeds in corn over a wide range of growth stages and are also effective on larger weeds when applications are delayed because of weather.

Excellent crop safety. Many of the herbicides labeled for corn have a narrow window of application in terms of plant size. Applications of Roundup agricultural herbicides to Roundup Ready corn can be made over a wide range of corn growth stages with excellent crop safety. Also, Roundup Ready corn has excellent tolerance to applications of Roundup agricultural herbicides over a wide range of weather conditions.

Control of herbicide-resistant weed species. Roundup agricultural herbicides will provide control of weeds resistant to other herbicides, namely the triazine and ALS-resistant weed species.

Greater flexibility and simplicity. The Roundup Ready corn system offers maximum flexibility to choose rotational crops. Because Roundup agricultural herbicides have no soil residual activity, farmers can choose from a large number of rotational crops to take

advantage of favorable markets. If a grower experiences a poor stand of corn due to hail or poor emergence, the grower will have the flexibility to replant with another crop.

Conservation tillage. The use of Roundup agricultural herbicides can increase adoption of conservation tillage systems that can provide a range of economic, agronomic and environmental benefits including reduced fuel costs, reduced machinery investment, conservation of soil moisture, decreased soil compaction, decreased soil erosion from wind and water, better water infiltration, improved surface water quality, enhanced carbon sequestration and increased population, and diversity of wildlife in and around fields (see Section I.B).

VIII.F.3. Impact on Insect Control Practices

Of the several insect species that can cause damage to corn plants, some of the most pernicious in the U.S. corn belt are larvae of the corn rootworm species, NCRW and WCRW (see Section I.B.2). These insects damage corn by feeding on the roots, reducing the ability of the plant to absorb water and nutrients from soil, and causing harvesting difficulties because of plant lodging (Riedell, 1990; Spike and Tollefson, 1991). Traditionally, these pests have been controlled by the use of nonselective insecticides such as organophosphate, carbamate and pyrethroids. Approximately 14 million acres of corn were treated with these insecticides in 2000 to control corn rootworm (Doane, 2001).

In 2003, Monsanto commercialized MON 863 to provide an alternative and more effective solution for the control of corn rootworm larvae based on biotechnology. MON 863 produces a CryBb1 protein that has activity only against the Coleopteran species of insects. Furthermore, the Cry3Bb1 protein has no toxic effects on mammals, is not likely to induce allergic or hypersensitive responses, and is less toxic than all of the major insecticides currently used to control corn rootworm damage. In granting the registration of MON 863, EPA assessed the potential benefits of corn rootworm protection technology by: a) comparing the efficacy of MON 863 with chemical control options for corn rootworm, b) evaluating the human health and environmental benefits compared to registered insecticides, c) estimating economic benefits to the grower, and d) estimating the chemical pesticide use reduction afforded by the adoption of MON 863. EPA made a determination that the registration of MON 863 was in the public interest and the benefits outweighed the risks (EPA, 2003).

This assessment indicates that the introduction of MON 88017 will result in a continuing reduction in the use of insecticides to control corn rootworm species. Because the Cry3Bb1 protein produced in MON 88017 has a narrow spectrum of activity that is confined to Coleopteran species, no significant impact is expected on the use of insecticides to control other species of insects in corn.

VIII.F.4. Impact on Crop Rotation Practices

Crop rotation is a key component for the production of corn. Crop rotation aids in the management of diseases, insects and weeds and increases organic matter and soil fertility. In addition, crop rotation allows growers to diversify farm production to minimize market

risks. For these reasons, rotational cropping in some form dominates most U.S. major crop production. According to USDA Economic Research Service (ERS) data based upon Agricultural Resource Management Study (ARMS) surveys, in their primary states of production, ≥ 98% of peanut, sunflower and potato acreage is cultivated in a crop rotation. Soybean and corn are only slightly lower at 92% and 84%, respectively (USDA ERS, 2003).

Crop rotation practices for field corn vary from state to state; however, there are similarities among states within certain growing regions. This section provides a detailed assessment of the crops planted in rotation with corn, including all states that grow more than 25,000 acres of corn annually, and accounting for 99+% of the total corn acreage. Planting data from the USDA National Agricultural Statistics Service for field crops (USDA-NASS, 2003a) and vegetable crops (USDA-NASS, 2002)[5] was used in this assessment. The rotational acreage for corn following corn for each state was obtained from AgroTrak surveys (Doane, 2003) and represents an average for the period 2001 to 2003. The data for the other rotational crops were obtained from personal communications with Monsanto technical personnel and university extension agronomists or specialists in each of the states. The rotational crop data were collected at the state level to provide the summary information at the regional and national levels:

- U.S. (Table VIII-12)
- Region and state:
  - Midwest: IL, IN, IA, KY, MI, MN, MO, NE, ND, OH, SD, WI (Table VIII-13)
  - Northeast: NY, PA, New England (Table VIII-14)
  - Mid-Atlantic: DE, MD, NJ, VA, WV (Table VIII-15)
  - Southeast: AL, AR, FL, GA, LA, MS, NC, SC, TN (Table VIII-16)
  - Great Plains: CO, KS, OK, TX, WY (Table VIII-17)
  - Northwest: ID, MT, OR, WA (Table VIII-18)
  - Southwest: AZ, CA, NM, UT (Table VIII-19)

The states of CT, NH, MA, ME, RI and VT, where the corn acreage is small, were grouped together into the New England states in the Northeast region.

Using the information obtained for rotational crops (columns B-G, J in Tables VIII-12 to VIII-19) an assessment was made as to what extent the acres planted with Roundup Ready corn (including MON 88017) would be rotated to another Roundup Ready crop (columns I, K in Tables VIII-12 to VIII-19). For the purposes of this assessment, the adoption rates used for Roundup Ready corn, soybeans and cotton in 2003 were obtained from the USDA-NASS June 30 Acreage Summary (USDA-NASS, 2003a). State percentages were used if they were provided, otherwise the national percentage was used in the calculations. Also, the percentages of Roundup Ready corn were assumed to be the total percentage of herbicide-tolerant corn plus herbicide-tolerant corn stacked with insect-resistance corn. Because the USDA-NASS report does not list the percentages of individual herbicide-resistant corn products, this is likely an overestimate for Roundup

[5] At the time this assessment was conducted, the 2003 acreage for vegetable crops was not available for all states.

Ready corn as it is likely that glufosinate-tolerant corn represents at least 3% of the corn acres (see Table VIII-7). For other Roundup Ready crops under development including alfalfa, sugar beet and wheat, a conservative adoption rate of 50% was assumed. Requests for the determination of deregulated status for these crops have been submitted to the USDA.

The results of this rotational crop assessment show that across the U.S. the majority of the corn acreage (61%) is rotated to soybeans (Column G, Table VIII-12). The second largest rotational crop following corn is corn (27%). Wheat, cotton, alfalfa hay, other hay, sorghum and oats are the next largest rotational crops following corn. However, they each represent less than 5% of the total corn acres. An additional 11 field crops and 11 vegetable crops have been identified as rotational crops following corn. On a regional basis, the highest rotations from corn to a following crop were as follows (Column G): soybean (70%) in the Midwest, corn (58%) in the Northeast, corn (41%) in the Mid-Atlantic, corn (32%) in the Southeast, corn (48%) in the Great Plains, corn (54%) in the Northwest, and, corn (53%) in the Southwest. In the Midwest, which represents over 80% of the corn acreage, the predominant rotation was from corn to soybean in most states, with the highest such rotation occurring in Missouri (83%).

In Table VIII-12, Column J provides the acreage of corn in each state as a percentage of the total rotational crop acreage to indicate whether corn is the primary crop preceding rotational crops. This percentage is 28.6% for the U.S., 2.3 to 40.1% on a regional basis, and 0.6 to 60.8% across all states.

Table VIII-12 also provides an assessment of the extent of Roundup Ready corn acreage likely to be rotated to another Roundup Ready crop in the U.S. Thus, across the U.S. (Column I), the rotations are predicted to be (highest to lowest): Roundup Ready soybean > Roundup Ready corn > Roundup Ready wheat > Roundup Ready cotton > Roundup Ready alfalfa > Roundup Ready sugar beet. On a regional basis, the predominant rotation from Roundup Ready corn is expected to be as follows: to Roundup Ready soybean in the Midwest, Mid-Atlantic, and Great Plains; to Roundup Ready corn in the Northeast and Northwest; to Roundup Ready wheat in the Southwest; and to Roundup Ready cotton in the Southeast. In the Midwest, Iowa is expected to have the highest number of acres rotated from Roundup Ready corn to other Roundup Ready crops, followed by Illinois and Minnesota.

The total Roundup Ready crop acreage rotated from Roundup Ready corn as a percentage of the total rotational crop acreage (Column K) is estimated to be 16.7% for the U.S. (Table VIII-12). This estimated percentage is highest in the Midwest region (24.6%) and lowest in the Northwest region (0.5%), and ranges from 0.2% (MT) to 35.7% (IA) across the corn-growing states. As discussed previously, these are likely overestimates given the assumption that other commercial herbicide-tolerant corn products are included in this calculation and the conservative estimates used for the market penetration of the Roundup Ready crops alfalfa, sugar beet and wheat under development.

These results lead to the conclusion that the percentage of rotation from Roundup Ready corn (including MON 88017) to other Roundup Ready crops is estimated to be <17%

across the U.S. and <25% in the Midwest. These levels of rotation are unlikely to have a significant impact on weed control and other agronomic practices currently used in corn.

Table VIII-12. U.S.: Rotational Crops Following Corn Including Roundup Ready crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
United States	**79062**	Corn	79062	26.6	21012	26.6	16%	3357		
		Soybeans	73653	65.2	48011	60.7	82%	39325		
		Wheat	57340	5.5	3125	4.0	50	1563		
		Alfalfa Hay	15904	9.0	1437	1.8	50	719		
		Other Hay	10630	7.7	819	1.0				
		Cotton	12914	11.8	1524	1.9	4%	968		
		Sorghum	8435	7.4	623	0.8	NA			
		Oats	1526	33.5	511	0.6	NA			
		Sugar Beets	1021	33.3	340	0.4	50	170		
		Sunflower	2097	15.6	327	0.4	NA			
		Barley	4826	5.0	242	0.3	NA			
		Peanuts	904	25.1	227	0.3	NA			
		Vegetables	1051	23.6	249	0.3	NA			
		Dry Beans	646	23.2	150	0.2	NA			
		Potatoes	824	16.4	135	0.2	NA			
		Millet	470	16.0	75	0.1	NA			
		Tobacco	160	69.4	111	0.1	NA			
		Rice	1916	0.4	9	0.01	NA			
		Rye	20	94.3	19	0.02	NA			
		Safflower	6	83.3	5	0.01	NA			
		Fallow	2611	4.3	112	0.1	NA			
			Total: 276017		**Total: 79062**			**Total: 46100**	**28.6%**	**16.7%**

Data in this table obtained by compiling regional data in Tables VIII-13 to VIII-19. See legend at the bottom of Table VIII-19 for explanation of calculations.

Table VIII-13. Midwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Region	**63880**	Corn	63880	22.1	14089	22.1	14.2%	2007		
		Soybeans	60070	74.5	44751	70.1	81.9%	36665		
		Wheat	17077	8.6	1477	2.3	50.0%	739		
		Alfalfa Hay	9140	12.9	1183	1.9	50.0%	592		
		Other Hay	5110	10.1	518	0.8	NA			
		Sorghum	1030	44.3	456	0.7	NA			
		Oats	900	36.2	326	0.5	NA			
		Sugar Beets	969	32.0	310	0.5	50.0%	155		
		Sunflower	1795	13.3	240	0.4	NA			
		Barley	2155	4.1	88	0.1	NA			
		Vegetables	359	51.5	185	0.3	NA			
		Dry Beans	512	23.9	122	0.2	NA			
		Potatoes	256	35.0	90	0.1	NA			
		Millet	170	26.5	45	0.1	NA			
			Total: 163425		**Total: 63880**			**Total: 40157**	**39.1%**	**24.6%**
IL	**11100**	Corn	11100	17.0	1887	17.0	5%	94		
		Soybeans	10600	84.3	8936	80.5	77%	6880		
		Wheat	800	16.7	133	1.2	50%	67		
		Alfalfa Hay	450	14.8	67	0.6	50%	33		
		Other Hay	350	12.7	44	0.4	NA			
		Sorghum	110	10.1	11	0.1	NA			
		Oats	60	37.0	22	0.2				
			Total: 23470		**Total: 11100**			**Total: 7075**	**47.3%**	**30.1%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-13 (cont'd). Midwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
IN	**5700**	Corn	5700	18.0	1026	18.0	8%	82		
		Soybeans	5400	84.4	4560	80.0	88%	4013		
		Wheat	450	12.7	57	1.0	50%	29		
		Alfalfa Hay	330	5.2	17	0.3	50%	9		
		Other Hay	320	5.3	17	0.3	NA			
		Vegetables*	32	71.3	23	0.4	NA			
		*Tomatoes, snap beans, melons, cucumbers	**Total: 12232**		**Total: 5700**			**Total: 4132**	**46.6%**	**33.8%**
IA	**12400**	Corn	12400	17.0	2108	17.0	12%	253		
		Soybeans	10400	95.4	9920	80.0	84%	8333		
		Alfalfa Hay	1380	21.6	298	2.4	50%	149		
		Other Hay	320	23.3	74	0.6	NA			
			Total: 24500		**Total: 12400**			**Total: 8735**	**50.6%**	**35.7%**
KY	**1230**	Corn	1230	34.0	418	34.0	19%	79		
		Soybeans	1120	41.7	467	38.0	76%	355		
		Wheat	480	71.8	344	28.0	50%	172		
			Total: 2830		**Total: 1230**			**Total: 607**	**43.5%**	**21.4%**
MI	**2300**	Corn	2300	26.0	598	26.0	17%	102		
		Soybeans	2100	61.3	1288	56.0	73%	940		
		Alfalfa Hay	750	23.0	173	7.5	50%	86		
		Other Hay	250	23.0	58	2.5	NA			
		Sugar Beets	179	89.9	161	7.0	50%	81		
		Dry Beans	200	11.5	23	1.0	NA			
			Total: 5779		**Total: 2300**			**Total: 1209**	**39.8%**	**20.9%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-13 (cont'd). Midwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
MN	**7100**	Corn	7100	19.0	1349	19.0	22%	297		
		Soybeans	7600	68.7	5219	73.5	79%	4123		
		Alfalfa Hay	1450	12.7	185	2.6	50%	92		
		Other Hay	750	13.3	99	1.4	NA			
		Sugar Beets	470	15.1	71	1.0	50%	36		
		Vegetables*	228	46.7	107	1.5	NA			
		Dry Beans	145	24.5	36	0.5	NA			
		Potatoes	60	59.2	36	0.5	NA			
		*Sweet Corn, peas	**Total: 17803**		**Total: 7100**			**Total: 4547**	**39.9%**	**25.5%**
MO	**2950**	Corn	2950	12.0	354	12.0	10%	35		
		Soybeans	4950	49.5	2449	83.0	83%	2032		
		Wheat	880	16.8	148	5.0	50%	74		
			Total: 8780		**Total: 2950**			**Total: 2141**	**33.6%**	**24.4%**
NE	**8000**	Corn	8000	39.0	3120	39.0	16%	499		
		Soybeans	4700	85.1	4000	50.0	86%	3440		
		Wheat	1800	22.2	400	5.0	50%	200		
		Sorghum	650	61.5	400	5.0	NA			
		Sugar Beets	40	50.0	20	0.25	50%	10		
		Dry Beans	160	37.5	60	0.75	NA			
			Total: 15350		**Total: 8000**			**Total: 4149**	**52.1%**	**27.0%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-13 (cont'd). Midwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
ND	**1450**	Corn	1450	38.0	551	38.0	19%	105		
		Soybeans	3100	17.3	537	37.0	74%	397		
		Wheat	8430	2.6	218	15.0	50%	109		
		Sugar Beets	280	20.7	58	4.0	50%	29		
		Sunflower	1330	1.1	15	1.0	NA			
		Barley	2100	2.1	44	3.0	NA			
		Potatoes	117	24.8	29	2.0	NA			
			Total: 16808		**Total: 1450**			**Total: 639**	**8.6%**	**3.8%**
OH	**3450**	Corn	3450	13.0	449	13.0	3%	13		
		Soybeans	4400	62.7	2760	80.0	74%	2042		
		Wheat	1000	6.9	69	2.0	50%	35		
		Alfalfa Hay	580	12.5	72	2.1	50%	36		
		Other Hay	770	13.0	100	2.9	NA			
			Total: 10200		**Total: 3450**			**Total: 2127**	**3.8%**	**20.8%**
SD	**4500**	Corn	4500	15.0	675	15.0	41%	277		
		Soybeans	4100	80.1	3285	73.0	91%	2989		
		Wheat	3025	3.0	90	2.0	50%	45		
		Alfalfa Hay	2600	2.1	54	1.2	50%	27		
		Other Hay	1900	1.9	36	0.8	NA			
		Sorghum	270	16.7	45	1.0	NA			
		Oats	460	9.8	45	1.0	NA			
		Sunflower	465	48.4	225	5.0	NA			
		Millet	170	26.5	45	1.0				
			Total: 17490		**Total: 4500**			**Total: 3338**	**25.7%**	**19.1%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-13 (cont'd). Midwest Region: Rotational Crops Following Corn Including Roundup Ready crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
WI	**3700**	Corn	3700	42.0	1554	42.0	11%	171		
		Soybeans	1600	83.3	1332	36.0	84%	1119		
		Wheat	212	8.7	19	0.5	50%	9		
		Alfalfa Hay	1600	19.9	318	8.6	50%	159		
		Other Hay	450	19.7	89	2.4	NA			
		Oats	380	68.2	259	7.0	NA			
		Barley	55	80.7	44	1.2	NA			
		Vegetables*	99	56.1	56	1.5	NA			
		Dry Beans	7	52.9	4	0.1	NA			
		Potatoes	81	32.0	26	0.7	NA			
		*Cabbage, carrots, cucumbers, snap beans	**Total: 8184**		**Total: 3700**			**Total: 1458**	**45.2%**	**17.8%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-14. Northeast Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acress.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Region	**2662**	Corn	2662	57.7	1535	57.7	19%	292		
		Soybeans	515	62.3	321	12.1	76%	244		
		Wheat	300	50.8	153	5.7	50%	76		
		Alfalfa Hay	1379	9.7	134	5.0	50%	67		
		Other Hay	2685	9.6	257	9.7	NA			
		Oats	256	65.4	167	6.3	NA			
		Barley	100	51.2	51	1.9	NA			
		Vegetables	110	22.5	25	0.9	NA			
		Rye	20	94.3	19	0.7	NA			
			Total: 8027		**Total: 2662**			**Total: 679**	**33.2%**	**8.5%**
NY	**1020**	Corn	1020	62.0	632	62.0	19%	120		
		Soybeans	145	91.4	133	13.0	76%	101		
		Wheat	125	40.8	51	5.0	50%	26		
		Alfalfa Hay	600	8.3	50	4.9	50%	25		
		Other Hay	1000	8.3	83	8.1	NA			
		Oats	85	60.0	51	5.0	NA			
		Vegetables*	102	20.0	20	2.0	NA			
		*Sweet corn, snap beans, cabbage	**Total: 3077**		**Total: 1020**			**Total: 271**	**33.1%**	**8.8%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-14 (cont'd). Northeast Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
PA	**1450**	Corn	1450	53.0	769	53.0	19%	146		
		Soybeans	370	50.9	189	13.0	76%	143		
		Wheat	175	58.0	102	7.0	50%	51		
		Alfalfa Hay	700	11.0	77	5.3	50%	38		
		Other Hay	1200	11.1	133	9.2	NA			
		Oats	140	77.7	109	7.5	NA			
		Barley	75	65.7	49	3.4	NA			
		Vegetables*	8	54.4	4	0.3	NA			
		Rye	20	94.3	19	1.3	NA			
		*Snap beans	**Total: 4138**		**Total: 1450**			**Total: 378**	**35.0%**	**9.1%**
New England	**192**	Corn	192	70.0	134	70.0	19%	26		
		Alfalfa Hay	79	8.5	7	3.5	50%	3		
		Other Hay	485	8.5	41	21.5	NA			
		Oats	31	24.8	8	4.0	NA			
		Barley	25	7.7	2	1.0	NA			
			Total: 812		**Total: 192**			**Total: 29**	**23.8%**	**3.6%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K**.**

Table VIII-15. Mid-Atlantic Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Region	**1315**	Corn	1315	41.4	544	41.4	19%	103		
		Soybeans	1317	36.4	480	36.5	76%	365		
		Wheat	468	46.3	217	16.5	50%	108		
		Barley	154	39.0	60	4.6	NA			
		Vegetables	29	50.3	15	1.1	NA			
			Total: 3283		**Total: 1315**			**Total: 576**	**40.1%**	**17.6%**
DE	**180**	Corn	180	38.0	68	38.0	19%	13		
		Soybeans	190	28.4	54	30.0	76%	41		
		Wheat	50	72.0	36	20.0	50%	18		
		Barley	25	50.4	13	7.0	NA			
		Vegetables*	17	52.9	9	5.0	NA			
		*Snap beans, lima beans	**Total: 462**		**Total: 180**			**Total: 72**	**39.0%**	**15.6%**
MD	**530**	Corn	530	38.0	201	38.0	19%	38		
		Soybeans	480	28.6	186	35.0	76%	141		
		Wheat	165	64.2	106	20.0	50%	53		
		Barley	45	82.4	37	7.0	NA			
			Total: 1220		**Total: 530**			**Total: 232**	**43.4%**	**19.0%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-15 (cont'd). Mid-Atlantic Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
NJ	**80**	Corn	80	58.0	46	58.0	19%	9		
		Soybeans	100	25.6	26	32.0	76%	19		
		Wheat	31	5.2	2	2.0	50%	1		
		Barley	4	20.0	1	1.0	NA			
		Vegetables*	12	46.7	6	7.0	NA			
		*Sweet corn, snap beans	**Total: 227**		**Total: 80**			**Total: 29**	**35.2%**	**12.8%**
VA	**480**	Corn	480	41.0	197	41.0	19%	37		
		Soybeans	530	38.0	202	42.0	76%	153		
		Wheat	210	34.3	72	15.0	50%	36		
		Barley	80	12.0	10	2.0	NA			
			Total: 1300		**Total: 480**			**Total: 227**	**36.9%**	**17.4%**
WV	**45**	Corn	45	69.0	31	69.0	19%	6		
		Soybeans	17	76.8	13	29.0	76%	10		
		Wheat	12	7.5	1	2.0	50%	0.5		
			Total: 74		**Total: 45**			**Total: 16**	**60.8%**	**22.0%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-16. Southeast Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Region	**3835**	Corn	3835	32.4	1244	32.4	19%	235		
		Soybeans	8631	9.5	817	21.3	79%	650		
		Wheat	1800	19.5	351	9.1	50%	175		
		Cotton	6340	16.8	1065	27.8	72%	762		
		Sorghum	205	4.1	8	0.2	NA			
		Peanuts	904	25.1	227	5.9	NA			
		Vegetables	133	2.8	4	0.1	NA			
		Tobacco	160	69.4	111	2.9	NA			
		Rice	1916	0.4	9	0.2	NA			
			Total: 23924		**Total: 3835**			**Total: 1822**	**16.0%**	**7.6%**
AL	**230**	Corn	230	51.0	117	51.0	19%	22		
		Soybeans	190	2.4	5	2.0	76%	3		
		Wheat	120	3.8	5	2.0	50%	2		
		Cotton	560	12.3	69	30.0	70%	48		
		Peanuts	190	18.2	35	15.0	NA			
			Total: 1290		**Total: 230**			**Total: 76**	**17.8%**	**5.9%**
AR	**350**	Corn	350	23.0	81	23.0	19%	15		
		Soybeans	2900	3.7	109	31.0	84%	91		
		Wheat	700	2.5	18	5.0	50%	9		
		Cotton	950	14.7	140	40.0	71%	99		
		Rice	1446	0.2	4	1.0	NA			
			Total: 6346		**Total: 350**			**Total: 215**	**5.5%**	**3.4%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-16 (cont'd). Southeast Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
FL	**85**	Corn	85	55.0	47	55.0	19%	9		
		Soybeans	11	7.7	1	1.0	76%	1		
		Wheat	20	4.3	1	1.0	50%	0.4		
		Cotton	100	12.8	13	15.0	70%	9		
		Peanuts	110	21.6	24	28.0	NA			
			Total: 326		**Total: 85**			**Total: 19**	**26.1%**	**5.8%**
GA	**370**	Corn	370	44.0	163	44.0	19%	31		
		Soybeans	180	2.1	4	1.0	76%	3		
		Wheat	380	1.9	7	2.0	50%	4		
		Cotton	1400	7.9	111	30.0	79%	88		
		Peanuts	500	16.3	81	22.0	NA			
		Vegetables*	133	2.8	4	1.0	NA			
		*All grown in the state	**Total: 2963**		**Total: 370**			**Total: 125**	**12.5%**	**4.2%**
LA	**500**	Corn	500	36.0	180	36.0	19%	34		
		Soybeans	900	5.6	50	10.0	76%	38		
		Wheat	150	6.7	10	2.0	50%	5		
		Cotton	550	45.5	250	50.0	61%	153		
		Sorghum	170	2.9	5	1.0	NA			
		Rice	470	1.1	5	1.0	NA			
			Total: 2740		**Total: 500**			**Total: 230**	**18.2%**	**8.4%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K**.**

Table VIII-16 (cont'd). Southeast Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
MS	**550**	Corn	550	31.0	171	31.0	19%	32		
		Soybeans	1360	10.9	149	27.0	89%	132		
		Cotton	1120	20.6	231	42.0	77%	178		
			Total: 3030		**Total: 550**			**Total: 342**	**18.2%**	**11.3%**
NC	**740**	Corn	740	25.0	185	25.0	19%	35		
		Soybeans	1430	15.5	222	30.0	76%	169		
		Cotton	850	17.4	148	20.0	77%	114		
		Peanuts	90	82.2	74	10.0				
		Tobacco	160	69.4	111	15.0				
			Total: 3270		**Total: 740**			**Total: 318**	**22.6%**	**9.7%**
SC	**320**	Corn	320	38.0	122	38.0	19%	23		
		Soybeans	480	28.7	138	43.0	76%	105		
		Cotton	250	19.2	48	15.0	70%	34		
		Peanuts	14	91.4	13	4.0	NA			
			Total: 1064		**Total: 320**			**Total: 161**	**30.1%**	**15.2%**
TN	**690**	Corn	690	26.0	179	26.0	19%	34		
		Soybeans	1180	12.0	141	20.5	76%	108		
		Wheat	430	72.2	311	45.0	50%	155		
		Cotton	560	9.9	55	8.0	70%	39		
		Sorghum	35	9.9	3	0.5	NA			
			Total: 2895		**Total: 690**			**Total: 335**	**23.8%**	**11.6%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-17. Great Plains Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Region	**6185**	Corn	6185	48.0	2966	48.0	19%	600		
		Soybeans	3120	52.6	1642	26.5	85%	1401		
		Wheat	26121	2.9	749	12.1	50%	375		
		Cotton	5816	7.6	440	7.1	45%	198		
		Sorghum	7050	2.2	158	2.6	NA			
		Sunflower	302	28.9	88	1.4	NA			
		Millet	300	10.0	30	0.5	NA			
		Fallow	2611	4.3	112	1.8	NA			
			Total: 51505		**Total: 6185**			**Total: 2574**	**12.0%**	**5.0%**
CO	**1000**	Corn	1000	69.0	690	69.0	19%	131		
		Wheat	2630	2.7	70	7.0	50%	35		
		Sorghum	350	17.1	60	6.0	NA			
		Sunflower	110	45.5	50	5.0	NA			
		Millet	300	10.0	30	3.0	NA			
		Fallow	2380	4.2	100	10.0				
			Total: 6770		**Total: 1000**			**Total: 166**	**14.8%**	**2.5%**
KS	**2900**	Corn	2900	42.0	1218	42.0	22%	268		
		Soybeans	2700	51.6	1392	48.0	87%	1211		
		Wheat	10300	2.0	203	7.0	50%	102		
		Sorghum	3700	1.6	58	2.0	NA			
		Sunflower	180	16.1	29	1.0	NA			
			Total: 19780		**Total: 2900**			**Total: 1581**	**14.7%**	**8.0%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-17 (cont'd). Great Plains Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
OK	**200**	Corn	200	47.0	94	47.0	19%	18		
		Soybeans	190	26.3	50	25.0	76%	38		
		Wheat	6400	0.9	56	28.0	50%	28		
			Total: 6790		**Total: 200**			**Total: 84**	**2.9%**	**1.2%**
TX	**2000**	Corn	2000	46.0	920	46.0	19%	175		
		Soybeans	230	87.0	200	10.0	76%	152		
		Wheat	6600	6.1	400	20.0	50%	200		
		Cotton	5816	7.6	440	22.0	45%	198		
		Sorghum	3000	1.3	40	2.0	NA			
			Total: 17646		**Total: 2000**			**Total: 725**	**11.3%**	**4.1%**
WY	**85**	Corn	85	52.0	44	52.0	19%	8		
		Wheat	191	10.7	20	24.0	50%	10		
		Sunflower	12	68.5	9	10.0	NA			
		Fallow	231	5.2	12	14.0	NA			
			Total: 519		**Total: 85**			**Total: 19**	**16.4%**	**3.6%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K**.**

Table VIII-18. Northwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Region	**455**	Corn	455	53.8	245	53.8	19%	46		
		Wheat	10150	0.2	24	5.2	50%	12		
		Alfalfa Hay	3800	1.2	47	10.4	50%	24		
		Other Hay	2200	0.7	15	3.4	NA			
		Oats	120	1.7	2	0.4	NA			
		Sugar Beets	52	57.7	30	6.6	50%	15		
		Barley	2340	0.8	18	3.9	NA			
		Vegetables	33	5.9	2	0.4	NA			
		Dry Beans	134	20.5	27	6.0	NA			
		Potatoes	566	7.8	44	9.8	NA			
			Total: 19850		**Total: 455**			**Total: 97**	**2.3%**	**0.5%**
ID	**200**	Corn	200	55.0	110	55.0	19%	21		
		Wheat	1240	0.8	10	5.0	50%	5		
		Alfalfa Hay	1200	3.3	40	20.0	50%	20		
		Other Hay	300	3.3	10	5.0	NA			
		Oats	120	1.7	2	1.0	NA			
		Barley	760	1.3	10	5.0	NA			
		Dry Beans	80	12.5	10	5.0	NA			
		Potatoes	360	2.2	8	4.0	NA			
			Total: 4260		**Total: 200**			**Total: 46**	**4.7%**	**1.1%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-18 (cont'd). Northwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
MT	**60**	Corn	60	10.0	6	10.0	19%	1		
		Wheat	5400	0.1	6	10.0	50%	3		
		Alfalfa Hay	1650	0.1	2	3.1	50%	1		
		Other Hay	1000	0.1	1	1.9	NA			
		Sugar Beets	52	57.7	30	50.0	50%	15		
		Barley	1200	0.5	6	10.0	NA			
		Dry Beans	16	56.3	9	15.0	NA			
			Total: 9378		**Total: 60**			**Total: 20**	**0.6%**	**0.2%**
OR	**65**	Corn	65	66.0	43	66.0	19%	8		
		Wheat	1110	0.2	3	4.0	50%	1		
		Alfalfa Hay	460	0.3	1	2.2	50%	1		
		Other Hay	590	0.3	2	2.8	NA			
		Barley	70	0.9	1	1.0	NA			
		Vegetables*	17	3.8	1	1.0	NA			
		Dry Beans	8	24.4	2	3.0	NA			
		Potatoes	43	30.2	13	20.0	NA			
		*Onions	**Total: 2363**		**Total: 65**			**Total: 10**	**2.8%**	**0.4%**
WA	**130**	Corn	130	66.0	86	66.0	19%	16		
		Wheat	2400	0.2	5	4.0	50%	3		
		Alfalfa Hay	490	0.8	4	3.1	50%	2		
		Other Hay	310	0.8	2	1.9	NA			
		Barley	310	0.4	1	1.0	NA			
		Vegetables*	16	8.1	1	1.0	NA			
		Dry Beans	30	21.7	7	5.0	NA			
		Potatoes	163	14.4	23	18.0	NA			
		*Onions	**Total: 3849**		**Total: 130**			**Total: 21**	**3.4%**	**0.5%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-19. Southwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
Region	**730**	Corn	730	53.4	390	53.4	19%	74		
		Wheat	1424	10.9	155	21.2	50%	77		
		Alfalfa Hay	1585	4.6	73	10.1	50%	37		
		Other Hay	635	4.5	29	3.9	NA			
		Cotton	758	2.5	19	2.6	42%	8		
		Sorghum	150	0.4	1	0.1	NA			
		Oats	250	6.0	15	2.1	NA			
		Barley	77	32.3	25	3.4	NA			
		Vegetables	387	4.9	19	2.6	NA			
		Safflower	6	83.3	5	0.7	NA			
			Total: 6002		**Total: 730**			**Total: 195**	**12.2%**	**3.3%**
AZ	**45**	Corn	45	35.0	16	35.0	19%	3		
		Alfalfa Hay	245	5.0	12	27.0	50%	6		
		Other Hay	45	5.0	2	5.0	NA			
		Barley	22	53.2	12	26.0	NA			
		Vegetables*	3	98.4	3	7.0	NA			
		*Chili peppers	**Total: 360**		**Total: 45**			**Total: 9**	**12.5%**	**2.5%**
CA	**500**	Corn	500	48.0	240	48.0	19%	46		
		Wheat	750	17.3	130	26.0	50%	65		
		Alfalfa Hay	1090	5.1	56	11.2	50%	28		
		Other Hay	470	5.1	24	4.8	NA			
		Cotton	700	2.1	15	3.0	30%	5		
		Oats	250	6.0	15	3.0	NA			
		Vegetables*	366	4.1	15	3.0	NA			
		Safflower	6	83.3	5	1.0	NA			
		*Tomatoes, melons	**Total: 4132**		**Total: 500**			**Total: 143**	**12.1%**	**3.5%**

See legend at the bottom of Table VIII-19 for explanation of data in Columns B-K.

Table VIII-19 (cont'd). Southwest Region: Rotational Crops Following Corn Including Roundup Ready Crops

All acreages are x1000 acres.

A	B	C	D	E	F	G	H	I	J	K
State	**Total Corn Acres[1]**	**Major Crops That Follow Corn In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Corn[2]**	**Rotational Crop Acres Following Corn[3]**	**Percent Rotational Crop of Total Corn[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Corn Acres Preceeding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
NM	**130**	Corn	130	81.0	105	81.0	19%	20		
		Wheat	500	2.3	12	9.0	50%	6		
		Alfalfa Hay	250	2.1	5	4.1	50%	3		
		Other Hay	120	2.1	2	1.9	NA			
		Cotton	58	6.7	4	3.0	70%	3		
		Sorghum	150	0.4	1	0.5	NA			
		Vegetables*	18	3.6	1	0.5	NA			
		*Chili peppers	**Total: 1226**		**Total: 130**			**Total: 31**	**10.6%**	**2.5%**
UT	**55**	Corn	55	52.0	29	52.0	19%	5		
		Wheat	174	7.6	13	24.0	50%	7		
		Barley	55	24.0	13	24.0	NA			
			Total: 284		**Total: 55**			**Total: 12**	**19.4%**	**4.2%**

Legend:

The data in Table VIII-12 were obtained by compilation of the regional data in Tables VIII-13 to VIII-19. The regional data in Tables VIII-13 to VIII-19 were obtained by compilation of all the state data within the region. Calculations for the individual columns are described below. Calculations specific to Table VIII-12 and the regional data in Tables VIII-13 to VIII-19 were as follows: a) Column G obtained by dividing Column F by Column B and multiplying by 100; b) Column H obtained by dividing Column I by Column F and multiplying by 100. NA = not applicable.

[1] Field crop planting data from USDA-NASS, 2003a and vegetable planting data from USDA-NASS, 2002 or USDA-NASS state websites (www.usda.gov/nass/sso-rpts.htm).

[2] Column E obtained by dividing Column F by Column D and multiplying by 100.

[3] Column F obtained by multiplying Column B by Column G.

[4] The percentages of corn following corn were obtained from the AgroTrak survery (Doane, 2003). Other rotational crop percentages are based on communications with local experts, University agronomist and Monsanto technical personnel.

[5] Roundup Ready rotational crop penetration rates for corn, soybeans and cotton based on 2003 planting data (USDA-NASS, 2003a). The percentages for Roundup Ready corn represent the total percentages for herbicide-tolerant corn plus herbicide-tolerant/insect-resistant stacked corn. Market penetration of Roundup Ready crops under development including alfalfa, sugar beet and wheat were assumed to be 50%.

[6] Column I obtained by multiplying Column F by Column H.

[7] Column J obtained by dividing Column B by Column D (total) and multiplying by 100.

[8] Column K obtained by dividing Column I (total) by Column D (total) and multiplying by 100.

VIII.G. Volunteer Management

Volunteer corn can occur in rotational crops planted following the harvest of corn grain; the occurence of volunteers is independent of whether the corn is conventional or has been improved through biotechnology. Volunteers in rotational crops do not occur with corn grown for silage (~9% of the US corn acres) since corn harvested for silage does not produce grain. In the warmer climates of the Southeast and Southwest, the occurrence of volunteer corn is rare because any corn grain remaining after harvest is likely to germinate in the fall and the resulting plants can usually be controlled by tillage or by freezing temperatures in the winter. In the Northern corn-growing regions, volunteer corn does not always occur in the rotational crop because of seed decomposition over the winter, efficient harvest procedures, and tillage prior to planting rotational crops.

The first step to manage volunteer corn in rotational crops is to minimize or reduce the potential for volunteers. The following practices should be implemented to reduce volunteer corn in rotational crops: 1) adjust harvest equipment to minimize the amount of corn grain lost in the field, 2) plant corn hybrids that reduce the extent of ear drop, 3) choose corn hybrids with superior stalk strength and reduced lodging, and 4) practice no-till production to significantly reduce the potential for volunteer growth in the rotational crop.

If volunteer corn does occur in subsequent crops, preplant tillage or in-crop cultivation is very effective in managing it. Table VIII-20 lists the herbicides labeled for the control of volunteer corn in the particular rotational crop. Assure II® (quizalofop), Fusilade® DX (fluazifop), Fusion® (fluazifop + fenoxaprop), Poast® (sethoxydim), and Select® 2EC (clethodim) provide effective postemergence control of volunteer corn in labeled crops. These products are labeled for use in eight field crops, including soybeans, cotton, sugar beet and alfalfa, and eleven vegetable crops identified as rotational crops for corn (see Table VIII-12). Small grains, sorghum, millet, tobacco, rice, and safflower do not have herbicides labeled for the control of volunteer corn. However, small grains (wheat, barley, oats and rye) are planted in the fall or very early spring and provide sufficient competition such that volunteer corn is rarely a weed-control issue in these crops. As in the past, no herbicidal solution is available for controlling volunteer corn in a corn-on-corn rotation. Although this is rarely a problem in corn production, preplant tillage and in-crop cultivation can be effective in minimizing the occurrence of such volunteers.

This assessment indicates that the introduction of MON 88017 will not impact the methods currently used for the control of volunteer corn.

® Assure II is a trademark of E.I. DuPont de Nemours, Inc.
® Fusilade and Fusion are trademarks of Syngenta Group Company.
® Poast is a trademark of BASF Corporation.
® Select is a trademark of Valent U.S.A. Corporation.

Table VIII-20. Herbicides Labeled for Control of Volunteer Corn in Labeled Rotational Crops

Crop[1]	Assure II	Fusilade DX	Fusion	Poast	Select 2EC
Soybeans	x	x	x	x	x
Hay				Alfalfa, Clover	Alfalfa, Clover
Cotton	x	x	x	x	x
Sugar Beets	x				
Sunflower					x
Peanuts				x	x
Dry Beans	x				x
Potatoes					x
Vegetables					
Cabbage					x
Cantaloupe					x
Carrots		x			x
Cucumbers					x
Peas, green	x				
Peppers, Chili					x
Onions					Bulbs only
Snap Beans	x				
Tomatoes					x
Watermelon					x

[1]An 'x' indicates that the herbicide is labeled for use in the particular crop.

VIII.H. Weed Resistance Management

The risk of weeds developing resistance, and the potential impact of resistance on the usefulness of an herbicide, vary greatly across different modes of action and are dependent on a combination of factors. Table VIII-21 lists the weed species that occur in corn that have been reported to show resistance to certain classes of herbicides. The majority of weeds listed are resistant to ALS inhibitor (class B) and Photosystem II inhibitor (class C1) herbicides. Only three weed species that occur in corn are known to be resistant to glyphosate, i.e., marestail or horseweed (*Conyza Canadensis*), rigid ryegrass (*Lolium rigidum*), and goosegrass (*Elusine indica*), and these are discussed in greater detail in Appendix I.

The use of glyphosate in a Roundup Ready corn system provides an additional mode of action for use in crops to control herbicide-resistant weeds. This reduces the selection pressure on these weeds and offers an alternative mode of action for controlling resistant weed populations. Glyphosate provides excellent control of ALS-resistant common ragweed and giant ragweed, triazine resistant lambsquarters and other weeds resistant to these herbicide classes.

Table VIII-21. Weeds Resistant to Herbicides Labeled for Use in Corn

Species	Common Name	Herbicide HRAC Class[1]
Abutilon theophrasti	Velvetleaf	C1
Amaranthus hybridus	Smooth Pigweed	B, C1
Amaranthus lividus	Livid Amaranth	B
Amaranthus palmeri	Palmer Amaranth	B, C1, K1
Amaranthus powellii	Powell Amaranth	B, C1, C2
Amaranthus retroflexus	Redroot Pigweed	B, C1, C2
Amaranthus rudis	Common Waterhemp	B, C1, E
Amaranthus tuerculatus	Tall Waterhemp	B
Ambrosia artemisiifolia	Common Ragweed	B, C1
Ammania auriculata	Redstem	B
Anmania coccinea	Long-Leaved Loosestrife	B
Ambrosia trifida	Giant Ragweed	B
Anthemis cotula	Mayweed Chamomile	B
Avena fatua	Wild Oat	A, B, K1, N, Z
Camelina microcarpa	Smallseed Falseflax	B
Centaurea solstitialis	Yellow Starthistle	O
Chenopodium album	Lambsquarters	B, C1
Convolvulus arvensis	Field Bindweed	O
Conyza Canadensis	Horseweed	B, C1, C2, D, G
Cyperus difformis	Smallflower Umbrella Sedge	B
Datura stramonium	Jimsonweed	C1
Daucus carota	Wild Carrot	O
Digitaria ischaemum	Smooth Crabgrass	A, O
Digitaria sanguinalis	Large Crabgrass	A
Echinochloa crus-galli	Barnyardgrass	C1, C2, N, O, A
Echinochloa oryzicola	Early Watergrass	A, N
Echinochloa phyllopogon	Late Watergrass	A, N
Eleusine indica	Goosegrass	D, K1
Helianthus annuus	Common Sunflower	B
Kochia scoparia	Kochia	B, C1, O
Lactuca serriola	Prickly Lettuce	B
Lolium multiflorum	Italian Ryegrass	A, B
Lolium perenne	Perennial Ryegrass	A, B
Lolium persicum	Persian Darnell	A
Lolium rigidum	Rigid Ryegrass	G
Phalaris minor	Little Seed Canary Grass	A
Poa anna	Annual Bluegrass	C1, C2, N, K1
Polygonum pensylvanicum	Pennsylvania Smartweed	C1
Polygonum persicaria	Ladysthumb	C1
Portulaca oleracea	Common Purslane	C1, C2

Table VIII-21 (cont'd). Weeds Resistant to Herbicides Labeled for Use in Corn

Species	Common Name	Herbicide HRAC Class[1]
Rottboellia exalta	Itchgrass	A
Salsola iberica	Russian Thistle	B
Sagittaria montevidensis	California Arrowhead	B
Scirpus mucronatus	Ricefield Bulrush	B
Senecio vulgaris	Common Groundsel	C1, C3
Setaria faberi	Giant foxtail	B, C1, A
Setaria lutescens	Yellow Foxtail (lutescens)	B
Setaria viridis	Green Foxtail	B, K1
Setaria viridis var. robustaalba Schreiber	Robust White Foxtail	A, B
Sida spinosa	Prickly sida	B
Sinapis arvensis	Wild Mustard	B
Solanum americanum	American Black Nightshade	D
Solanum ptycanthum	Eastern Black Nightshade	B
Sonchus asper	Spiny Sowthistle	B
Sorghum bicolor	Shattercane	B
Sorghum halepense	Johnsongrass	A, K1, B
Xanthium strumarium	Common Cocklebur	B, Z

[1]HRAC = Herbicide Resistance Action Committee. The herbicide classes are defined in Table VIII-8.

VIII.I. Insect Resistance Management

A critical component for the long-term use of genetically-enhanced *B.t.* crops containing insecticidal proteins is to implement Insect Resistance Management (IRM) programs to prevent or delay the onset of resistance in the target insect species. Research by industry as well as academic scientists over the past decade has improved understanding and gained broad agreement for the major elements of IRM plans for *B.t.* crops.

The core element of an IRM plan is the use of a refuge to ensure an adequate population of susceptible insects of the target species is available to mate with any resistant insects that survive exposure to the *B.t.* protein produced by the crop. This refuge may include wild host plants, other crops, or non-*B.t.* plantings of the crop in question.

Monsanto has developed an interim IRM plan for MON 863 (EPA, 2003) to delay the onset of resistance to the Cry3Bb1 protein in susceptible coleopteran species such as western, northern and Mexican corn rootoworms. This plan requires that a structured refuge of at least 20% non-Cry3Bb1 corn must be planted within or adjacent to the MON 863 corn field to ensure adequate mixing of insects between the refuge and the insect-protected corn field. Because the Cry3Bb1 protein in MON 88017 is expressed at a similar level as the Cry3Bb1 protein in MON 863 during the early stages of corn plant development (V2-V8) when corn rootworm larvae are exposed to the proteins, the proposed IRM plan for MON 88017 is identical to that for MON 863.

VIII.J. Overall Environment and Agronomic Practices Conclusions

An environmental assessment of MON 88017 was conducted to assess the impact of the introduced CP4 EPSPS and Cry3Bb1 proteins, the potential for gene flow, and the weediness potential of MON 88017.

In the comparative studies conducted between MON 88017 and a conventional control, dormancy, germination, phenotypic and pollen morphology characteristics were evaluated for changes that would impact plant pest potential, and in particular, plant weed potential. Furthermore, extensive postharvest monitoring of field trial plots planted with MON 88017 conducted under USDA-APHIS notifications did not reveal any differences in survivability or persistence relative to other corn. These data demonstrate that MON 88017 is no more likely to become a weed than conventional corn.

An assessment of the potential for gene flow indicates that MON 88017 is expected to be similar to conventional corn. Thus pollen-mediated gene flow is expected to occur only with cultivated *Zea* species but not with wild relatives such as *Tripsacum* species that occur in some regions of the U.S., but do not hybridize readily with cultivated corn.

An evaluation of the CP4 EPSPS protein based on its similarity to natural EPSPS proteins that are ubiquitous in the enviroment and lack of toxicity in laboratory investigations of

Roundup Ready crops demonstrates that the CP4 EPSPS protein produced in MON 88017 will have a minimal impact on the environment.

The environmental assessment of the Cry3Bb1 protein produced in MON 88017 is based on the extensive ecological assessment previously conducted for MON 863. The previous assessment included the testing of several Cry3Bb1 protein variants on representative species of bird, fish and terrestrial nontarget insects, estimation of soil dissipation times, and evaluation of the impact on endangered species and soil microorganisms. Theoretical estimates of margins of exposure (MOEs) for NTOs exposed to the Cry3Bb1 protein produced in MON 88017 show that they are in a similar range to those observed for MON 863. Thus, it is concluded that the Cry3Bb1 protein produced in MON 88017 poses no adverse risk to nontarget organisms and the environment.

An assessment of current corn agronomic practices determined that the introduction of MON 88017 will not impact cultivation practices and the managment of insects and diseases other than the control of corn rootworm larvae. The use of MON 88017 will allow for the broadspectrum control of grass, broadleaf and perennial weeds by over-the-top applications of Roundup agricultural herbicides. No impact is expected in the management of volunteer corn because there are a number of herbicides labeled for rotational crops that have a mode of action that is different than that of glyphosate.

IX. Adverse Consequences of Introduction

Monsanto knows of no study results or observations associated with MON 88017 that would be anticipated to result in adverse environmental consequences from its introduction. MON 88017 provides tolerance to applications of Roundup agricultural herbicides and protection from feeding damage caused by corn rootworm species. As demonstrated by field results and laboratory tests, the only phenotypic difference between MON 88017 and conventional corn is the presence of the CP4 EPSPS and Cry3Bb1 proteins. MON 88017 expresses the same CP4 EPSPS protein as in Roundup Ready corn NK603 and in other commercial Roundup Ready crops including soybean, cotton, and canola, and expresses the same Cry3Bb1 protein as in YieldGard Rootworm corn (MON 863). No adverse consequences as a result of the introduction and commercial use of Roundup Ready corn NK603 and YieldGard Rootworm corn (MON 863) have been reported to date.

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APPENDICES

Appendix A: Materials and Methods for Molecular Characterization

Molecular analysis was performed using genomic DNA isolated from MON 88017 in order to characterize the integrated DNA. MON 88017 genomic DNA was analyzed by Southern blot analysis for the number of integration sites, the number of copies of the integrated DNA at each locus, the integrity of the inserted gene cassettes, the presence or absence of plasmid backbone sequence and to determine the stability of the introduced DNA across multiple generations. Additionally, PCR amplification and DNA sequencing were used to confirm the 5' and 3' insert-to-plant junctions, confirm the organization of the elements within the insert, and determine the complete DNA sequence of the integrated DNA.

A.1. Test Substance

The test substance was MON 88017. Genomic DNA was isolated from the grain of MON 88017 [generation (LH198BC0F_1 x LH59) F_2]. Additional test substances from the breeding tree of MON 88017 (Figure V-12) were used in the insert stability analysis. For this analysis, DNA was isolated from the following seed lots: GLP-0203-12151-S, GLP-0203-12152-S, GLP-0203-12153-S, GLP-0203-12154-S, GLP-0203-12155-S, and GLP-0203-12156-S.

A.2. Control Substance

The control substance was a conventional corn of similar genetic background of the test substance. An additional control substance, the conventional corn line LH198, was used in the insert stability analysis.

A.3. Reference Substances

The primary reference substance was the plasmid PV-ZMIR39 that was used in transformation to produce MON 88017. For Southern blot analyses of corn genomic DNA, digested plasmid PV-ZMIR39 (approximately 0.5 and 1 genome copy equivalents) was mixed with digested DNA from the control substance and separated by electrophoresis on agarose gels. Additional reference standards included the 1 kb DNA Extension Ladder and 500 bp DNA Ladder from Life Technologies which were used for size estimations on Southern blots and PCR agarose gels, respectively.

A.4. Characterization of Test, Control and Reference Substances

Event-specific PCR assays were used to confirm the identity of the test and control substances. The stability of the DNA isolated from the test and control substances was determined in each Southern analysis by observation of the digested DNA sample on an ethidium bromide stained agarose gel. The identity of the test and control substances used in the insert stability analysis was confirmed by the molecular fingerprints generated from the Southern blot analyses.

A.5. Genomic DNA Isolation for Southern Blot Analyses

Genomic DNA from the test and control substances was extracted from corn grain by first processing the grain to a fine powder. Approximately 6 g of the processed grain were transferred to a 50 ml conical tube, then ~16 ml of CTAB extraction buffer [1.5% (w/v) CTAB, 75 mM Tris-HCl pH 8.0, 100 mM EDTA pH 8.0, 1.05 M NaCl, and 0.75% (w/v) PVP (MW 40,000)] and 8 µl of RNase (10 mg/ml, Roche) were added to the processed grain. The samples were incubated at 65°C for 30-60 minutes with intermittent mixing and then allowed to cool to room temperature. Approximately 15 ml of chloroform:isoamyl alcohol (24:1 (v/v)) were added to the samples. The suspension was mixed for 5 minutes and the two phases separated by centrifugation at ~16,000 x *g* for 5 minutes at room temperature. The aqueous (upper) layer was transferred to a clean 50 ml conical tube. Approximately 1/10 volume (~1.5 ml) of 10% CTAB buffer [10% (w/v) CTAB and 0.7 M NaCl] and an equal volume of chloroform:isoamyl alcohol [24:1 (v/v)] were added to the aqueous phase, which was then mixed for 5 minutes. The samples were centrifuged at ~16,000 x *g* for 5 minutes to separate the phases. The aqueous (upper) layer was removed, mixed with an equal volume (~15 ml) of CTAB precipitation buffer [1% (w/v) CTAB, 50 mM Tris pH 8.0, and 10 mM EDTA pH 8.0] and allowed to stand at room temperature for 1-2 hours. The samples were centrifuged at ~10,000 x *g* for 10 minutes at room temperature to pellet the DNA. The supernatant was discarded, and the pellet was dissolved in approximately 2 ml of high salt TE buffer (10mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, and 1 M NaCl). Gentle swirling at 37°C was performed to aid in dissolution of the pellet. If necessary, samples were centrifuged at ~23,000 x *g* at room temperature for 2 minutes to pellet and remove debris. Approximately 1/10 volume (~150 µl) of 3 M NaOAc (pH 5.2) and 2 volumes (~4 ml relative to the supernatant) of chilled 100% ethanol were added to precipitate the DNA. The precipitated DNA was spooled into a microcentrifuge tube containing 70% ethanol. The DNA was pelleted in a microcentrifuge at maximum speed (~14,000 rpm) for ~5 minutes, vacuum-dried, and re-dissolved in TE buffer (pH 8.0). The DNA was then stored in a 4°C refrigerator.

A.6. Quantitation of Genomic DNA

The quantity of the DNA in the samples was determined by using a Hoefer DyNA Quant 200 Fluorometer with Roche molecular size marker IX as a DNA calibration standard.

A.7. Restriction Enzyme Digestion of Genomic DNA

Approximately 20 µg of genomic DNA from MON 88017 and 10 µg of genomic DNA from the control substance were used for restriction enzyme digestions. For the insert stability analysis, approximately 10 µg of genomic DNA from the test substance were used. Overnight digests were performed at 37°C in a total volume of ~500 µl using 100 units of the appropriate restriction enzyme. After digestion, the samples were precipitated by adding 1/10 volume (50 µl) of 3 M NaOAc (pH 5.2) and 2 volumes (1 ml relative to the original digest volume) of 100% ethanol, followed by incubation in a

-20°C freezer for at least 30 minutes. The digested DNA was pelleted at maximum speed in a microcentrifuge, washed with 70% ethanol, dried, and re-dissolved in TE buffer.

A.8. DNA Probe Preparation for Southern Blot Analyses

DNA probe templates outlined in Figures V-1a and V-1b were prepared by PCR amplification of plasmid PV-ZMIR39. Approximately 25 ng of each probe template (except the NOS 3' and tahsp17 3' polyadenylation sequences) were labeled with ^{32}P-dCTP (6000 Ci/mmol) by a random priming method (RadPrime DNA Labeling System, Life Technologies). The NOS 3' and tahsp17 3' polyadenylation sequences were labeled by PCR using 25 ng of DNA probe template in the following manner: sense and antisense primers specific to the template (0.25 µM each); 1.5 mM $MgCl_2$; 3 µM each of dATP, dGTP and dTTP; ~100 µCi of ^{32}P-dCTP (6000 Ci/mmol); and 2.5 Units of *Taq* DNA polymerase in a final volume of 20 µl. The cycling conditions were as follows: 1 cycle at 94^{o}C for 3 minutes; 2 cycles at 94^{o}C for 45 seconds, 52^{o}C for 30 seconds, 72^{o}C for 2 minutes; and 1 cycle at 72^{o}C for 10 minutes. All radiolabeled probes were purified using a Sephadex G-50 column (Roche).

A.9. Southern Blot Analyses of Genomic DNA

Southern blot analyses were performed based on a method described by Southern (Southern, 1975). The samples of DNA digested with restriction enzymes were separated, based on size, using 0.8% agarose gel electrophoresis. A long run and short run were performed during this gel electrophoresis. The ~20 µg samples of digested MON 88017 DNA were divided in half for loading with ~10 µg on the long run and ~10 µg on the short run. The long run enabled greater separation of higher molecular weight DNAs while the short run allowed smaller molecular weight DNAs to be retained on the gel. The long run samples were loaded onto the gel and typically separated by electrophoresis for ~3-5 hours at 60-70 volts. The short run samples were then loaded in adjacent lanes on the same gel and separated by electrophoresis for ~14-17.5 additional hours at 30-35 volts. In some cases, the long run samples were loaded onto the gel and separated by electrophoresis for 15-17 hours at 30-35 volts. The short run samples in these cases were then loaded in adjacent lanes on the same gel and separated by electrophoresis for an additional 3.5-4.5 hours at 75-85 volts. For the insert stability analysis, a long run and short run were not performed. In this case, all samples were loaded onto the gel and separated by electrophoresis for 14.5 hours at 35 V and then for 3 hours at 60 V.

After electrophoresis, the gels were stained in 1.5 µg/ml ethidium bromide for 10-15 minutes and photographed. After photographing, the gels were placed in a depurination solution (0.125 N HCl) for 10-15 minutes followed by a denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 30-40 minutes and then a neutralizing solution (0.5 M Tris-HCl pH 7.0, 1.5 M NaCl) for 30-40 minutes. The gels were then placed in a 20X SSC buffer for 5-15 minutes. The DNA from the agarose gels was transferred to Hybond-N nylon

membranes (Amersham) using a Turboblotter™ (Schleicher & Schuell). The DNA was allowed to transfer overnight (using 20X SSC as the transfer buffer) and covalently cross-linked to the membrane with a UV Stratalinker® 1800 (Stratagene) using the auto-crosslink setting. The blots were prehybridized for 0.5-7 hours at 60-65°C in an aqueous solution of 250 mM $Na_2HPO_4 \bullet 7H_2O$, 7% SDS, and 0.1 mg/ml tRNA. Hybridization with the radiolabeled probe was performed in fresh prehybridization solution for 15-28 hours at 60°C (NOS 3' and tahsp17 3' polyadenylation sequence probes) or 65°C (all other probes). Membranes were washed in an aqueous solution of 0.1% SDS and 0.1X SSC for four 15-20 minute periods at the hybridization temperature using fresh solution for each of the four washes. Multiple exposures of the blots were then generated using Kodak Biomax™ MS-2 film in conjunction with one Kodak Biomax MS intensifying screen at approximately -80°C.

A.10. PCR Analyses

Overlapping PCR products were generated that span the insert in MON 88017 (Products A-G, Figures V-14 and V-15). These products were sequenced to verify the sequence of the insert in MON 88017 and to verify the sequence of the genomic DNA flanking the 5' and 3' ends of the insert. The PCR analyses were conducted using 50 ng of genomic DNA or 0.5 ng of plasmid PV-ZMIR39 positive control DNA template in a 50 µl reaction volume containing a final concentration of 1.5 mM $MgCl_2$, 0.4 µM of each primer, 200 µM each dNTP, and 2.5 units of *Taq* DNA polymerase. The specific polymerase used to amplify Products A, B, and D-G was REDTaq™ DNA Polymerase (Sigma). HotStarTaq™ DNA Polymerase (Qiagen) was used to amplify Product C. The amplification of Products A, B, and D-G was performed under the following cycling conditions: 94°C for 3 minutes; 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 10 minutes. The amplification of Product C was performed under the following cycling conditions: 94°C for 15 minutes; 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 10 minutes. The PCR products were separated on 1.2 % agarose gels and visualized by ethidium bromide staining. Following electrophoresis, PCR products generated from MON 88017 were excised from the gel and purified using the QIAquick® Gel Extraction Kit (Qiagen). The purified products were sequenced with the initial PCR primers as well as primers designed internal to the amplified sequences. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry.

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Appendix B: Materials, Methods, and Results for the Characterization of the CP4 EPSPS Protein

The CP4 EPSPS protein was purified from the grain of MON 88017 as well as from *E. coli* culture. A panel of analytical tests was used to identify, characterize and compare the plant- and *E. coli*-produced CP4 EPSPS proteins: (1) Western blot analysis and densitometry, (2) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry, (3) matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS), (4) N-terminal sequence analysis, (5) glycosylation analysis, and (6) CP4 EPSPS activity assay. The materials, methods and the results are described below.

B.1. Materials and Methods

B.1.a. Plant-Produced CP4 EPSPS Protein

The CP4 EPSPS protein was isolated from MON 88017 grain. The identity of the corn grain sample containing MON 88017 was confirmed using event-specific PCR analysis. The CP4 EPSPS protein was stored in a –80 °C freezer in a buffer solution (50 mM Tris-HCl, pH 7.5, 50 mM KCl, and 2 mM DTT) at a total protein concentration of 0.5 mg/mL.

B.1.b. *E. coli*-Produced CP4 EPSPS Protein

The *E. coli*-produced CP4 EPSPS protein (APS lot 20-100015) was used as a reference for the functional activity assays, glycosylation analysis, and Western blot analysis. The CP4 EPSPS protein was stored in a –80 °C freezer in a buffer solution (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine-HCl, and 25% [v/v] glycerol) at a total protein concentration of 3.8 mg/mL.

B.1.c. Assay Controls

Protein molecular weight standards were used to calibrate SDS-polyacrylamide gels and verify protein transfer to PVDF membranes. A peptide mixture was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. NIST BSA was used for MALDI-TOF intact mass analysis. Transferrin was used as the positive control in glycosylation analysis. An analytical reference protein (10 pmole β-lactoglobulin) was analyzed before and after the test proteins to verify that the N-terminal sequencer met acceptable performance criteria for repetitive yield and sequence identity. Phenylthiohydantoin amino acid standards were used during N-terminal sequencing analysis. NIST BSA, NIST AA Standards, and norvaline were used as standards for amino acid analysis.

B.1.d. Protein Purification

The CP4 EPSPS protein was purified from extracts of ground MON 88017 grain using a combination of anion exchange chromatography, ammonium sulfate fractionation, hydrophobic interaction chromatography, and affinity chromatography.

Defatted corn grain was extracted with a 50 mM Tris-HCl buffer, pH 8.0 [1:10 (w/v) grain to buffer ratio]. The extract was clarified by centrifugation and the supernatant was concentrated, dialyzed into 50 mM sodium carbonate/bicarbonate, 1 mM EDTA buffer, and applied to a Q-sepharose Fast Flow anion exchange column. The column was washed with 50 mM sodium carbonate/bicarbonate, 1 mM EDTA buffer and the eluate was collected. The eluate was clarified by centrifugation and the proteins were precipitated with ammonium sulfate (70% [w/v]). The ammonium sulfate pellet was re-suspended in 50 mM Tris-HCl, pH 7.5, 1.25 M ammonium sulfate buffer and an aliquot was applied to a Source 15PHE hydrophobic interaction column. Proteins bound to the column were eluted with a linear ammonium sulfate gradient (1.25 M to 0 M). Fractions were pooled based on immunoreactivity with anti-CP4 EPSPS antibodies on lateral flow strips, concentrated, and the buffer was exchanged to 25 mM Bis-Tris Propane, pH 6.5, containing protease inhibitors. The sample was applied to a Source 15 Q anion exchange column. The column was washed with 25 mM Bis-Tris propane, 1 mM DTT, 1 mM benzamidine, 0.5 mM PMSF, 10% (v/v) glycerol, pH 6.5 buffer. Proteins bound to the column were eluted with a linear sodium chloride gradient (0 M to 0.5 M NaCl). Fractions containing CP4 EPSPS protein were pooled based on immunoreactivity with anti-CP4 EPSPS antibodies on lateral flow strips, concentrated, and the buffer was exchanged to 50 mM MES, pH 5.85, 2 mM DTT. The sample was applied to a cellulose phosphate column for affinity chromatography. The column was washed with 50 mM MES, pH 5.85, 2 mM DTT. CP4 EPSPS protein was eluted from the column with 0.5 mM PEP, 0.5 mM S-3-P, 50 mM MES pH 5.85, 2 mM DTT buffer. Fractions were pooled based on immunoreactivity with CP4 EPSPS antibodies on lateral flow strips and SDS-PAGE analysis. The protein was concentrated and the buffer was exchanged to 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM DTT.

B.1.e. Total Protein Concentration Determination

The total protein concentration of the plant-produced CP4 EPSPS protein sample was determined using amino acid analysis. Aliquots of the sample were subjected to vapor phase acid hydrolysis followed by amino acid analysis on a Hitachi L-8800 amino acid analysis system. Amino acids were detected using post-column ninhydrin derivatization. Each protein sample was analyzed in triplicate. Total protein concentration was calculated as an average of the triplicate analysis.

B.1.f. Western Blot Analysis

Prior to Western blot analysis, proteins were subjected to SDS-PAGE and transferred to a PVDF membrane. Aliquots of the plant- and *E. coli*-produced CP4 EPSPS proteins were diluted and mixed with loading buffer to a final concentration of 1× loading buffer

(62.5 mM Tris, pH 6.8, 2% [w/v] SDS, 10% [v/v] glycerol, 2.5% [v/v] 2-mercaptoethanol, 0.005% [w/v] bromophenol blue). Samples were heated at 99-100 °C for 5 min prior to loading onto a Tris-glycine buffered 4-20% gradient polyacrylamide gel. Bio-Rad (Hercules, California) pre-stained broad range molecular weight markers (10 µL/lane) were included on the gel to verify transfer of proteins to the PVDF membrane. Electrophoresis was performed at a constant voltage of 160 V for 80 min. Following electrophoresis, samples were transferred to a 0.45 µm PVDF membrane for 90 min at a constant current of 300 mA in transfer buffer [12 mM Tris, 96 mM glycine, 20% (v/v) methanol, pH 8.3].

For Western blot analysis, the following steps were performed. The membrane was blocked by incubation in 5% (w/v) NFDM in 1× PBST for 18-20 h. Previously characterized antibodies (goat anti-CP4 EPSPS serum, lot 6844572) were diluted (1:2000) in 1% (w/v) NFDM in PBST and incubated with the membrane for 1 h. Excess serum was removed by washing with PBST four times for 10 min each. The membrane was then incubated with a 1:10,000 dilution of anti-goat IgG, HRP conjugated antibody in 1% (w/v) NFDM in PBST for 1 h. Excess antibody was removed by washing with PBST four times for 10 min each. All incubations were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (Amersham Pharmacia, Uppsala, Sweden) and the membrane was exposed (1 min, 30, 15, 10, 5, and 1 sec) to Hyperfilm ECL high performance chemiluminescence film (Amersham Pharmacia). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

Image analysis of immunoreactive bands on films was conducted using a Bio-Rad model GS-710 calibrated imaging densitometer equipped with Quantity One software version 4.3.0. The level of signal for the principal bands corresponding to the CP4 EPSPS protein detected in each lane was measured as a band contour quantity (average band OD × band area in mm^2).

The percent difference in immunoreactivity between the plant- and *E. coli*-produced proteins was calculated. The proteins were considered to have equivalent immunoreactivity if they differed by ≤ 10%.

B.1.g. N-terminal Sequence Analysis

Prior to N-terminal sequence analysis, the plant-produced CP4 EPSPS protein sample was separated using SDS-PAGE and transferred to a PVDF membrane. An aliquot of plant-produced CP4 EPSPS protein was diluted and mixed with loading buffer to a final concentration of 1× loading buffer. The sample was heated to ~102 °C for 4 minutes prior to loading onto a Tris-glycine buffered 4-20% gradient polyacrylamide gel. Bio-Rad pre-stained broad range molecular weight markers were included on the gel to verify transfer of the proteins to the membrane. Electrophoresis was performed at a constant voltage of 125 V for 100 min. Following electrophoresis, the gel was soaked for 60 minutes in CAPS transfer buffer and the proteins were transferred to a PVDF membrane (0.2 µm pore size) for 90 minutes at a constant current of 300 mA. Protein bands were

stained with Coomassie Brilliant Blue G stain (Bio-Rad) and then the blot was de-stained in a solution of 10% (v/v) acetic acid, 40% (v/v) methanol. Protein bands corresponding to CP4 EPSPS protein (~ 45 kDa) and two other protein bands found at ~48 kDa and ~55 kDa were excised from the membrane and sequenced.

N-terminal sequence analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise HT Protein Sequencing System (Foster City, CA) with 140C Microgradient system and 785 Programmable Absorbance Detector and Procise Control Software (version 1.1a) were used. Chromatographic data were collected using Atlas software (version 3.59a). A PTH-amino acid standard mixture was used to chromatographically calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as percent peak resolution and relative amino acid chromatographic retention times. A control protein (10 picomole β-lactoglobulin) was analyzed before and after the test substance protein bands to verify that the sequencer met acceptable performance criteria for repetitive yield and sequence identity.

B.1.h. MALDI-TOF MS Tryptic Mass Map Analysis

B.1.h.1. In-Gel Trypsin Digestion and Sample Preparation

Prior to the generation of tryptic fragments for MALDI-TOF mass spectroscopy, aliquots of the plant-produced CP4 EPSPS protein in loading buffer were separated by electrophoresis on a Tris-glycine buffered 4-20% gradient polyacrylamide gel. Prior to loading, the plant-produced CP4 EPSPS protein samples were heated at ~102 °C for 4 min. Bio-Rad broad range molecular weight markers were included on the gel. Electrophoresis was performed at a constant voltage of 125 V for 100 min. Following electrophoresis, the gel was stained for ~1 h with Coomassie Brilliant Blue G stain and destained for ~3 h in a solution containing 25% (v/v) methanol.

The band that migrated to ~45 kDa was excised, de-stained, reduced, alkylated, and subjected to an in-gel trypsin digest (Williams et al., 1997). Each gel fragment was destained by incubation in 100 µL of 40% (v/v) methanol and 10% (v/v) glacial acetic acid in a microfuge tube. Following destaining, gel fragments were incubated in 100 µL of 100 mM ammonium bicarbonate buffer for 30 min at room temperature. Proteins were reduced in 100 µL of 10 mM dithiothreitol solution for two hours at 37°C. Proteins were then alkylated by the addition of 100 µL of buffer containing 100 mM iodoacetic acid. The alkylation reaction was allowed to proceed at room temperature for 20 min in the dark. Gel fragments were incubated in 100 µL of 100 mM ammonium bicarbonate buffer for 30 min at room temperature. Acetonitrile (100 µL) was added to each microfuge tube and the reactions were incubated at room temperature for an additional 30 min. This procedure was repeated two additional times to remove the reducing and alkylating agents from the gel fragments. The gel bands were dried in a SpeedVac concentrator, rehydrated, and the protein digested in the gel overnight at 37 °C with 50 µL 25 mM ammonium bicarbonate solution containing 33 µg/mL trypsin. Digested peptides were extracted for one hour each at room temperature with 50 µL 70% (v/v) acetonitrile

containing 0.1% (v/v) trifluoroacetic acid (TFA). The supernatant from each extraction sample was combined and dried in a SpeedVac concentrator. The extraction, pooling, and drying were repeated two additional times, and the final dried material was stored overnight in a 4 °C refrigerator followed by reconstitution in 5 μL of 0.1% (v/v) TFA.

A portion of the digested sample was desalted (Bagshaw et al., 2000) using Millipore (Bedford, MA) $ZipTip_{C18}$ pipette tips. Prior to desalting, the tips were wetted with methanol and equilibrated with 0.1% (v/v) TFA. A sample (4.7 μL) was applied to a ZipTip and eluted with 5 μL of Wash 1 (0.1% [v/v] TFA), 5 μL of Wash 2 (20% [v/v] acetonitrile containing 0.1% [v/v] TFA), 5 μL of Wash 3 (50% [v/v] acetonitrile containing 0.1% [v/v] TFA), and finally with 5 μL of Wash 4 (90% [v/v] acetonitrile containing 0.1% [v/v] TFA).

B.1.h.2. MALDI-TOF Instrumentation and Mass Analysis

Mass spectral analysis was performed using an Applied Biosystems Voyager DE-Pro Biospectrometry Workstation MALDI-TOF instrument with the supplied Data Explorer software (version 4.0). Calibration of the mass spectrometer was performed using an external peptide mixture from a Sequazyme Peptide Mass Standards kit (Applied Biosystems). Samples (0.3 μL) from each desalting step, as well as a sample of the solution prior to desalting, were co-crystallized with 0.75 μL of α-cyano-4-hydroxy cinnamic acid on the analysis plate. All samples were analyzed from 500 to 5000 Daltons in reflector mode using 150 laser shots per mass spectrum at a laser intensity setting of 3100 (a unit-less MALDI-TOF instrument specific value). Protonated (MH+) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical trypsin digest of the expected protein sequence. The amino acid sequence of the plant-produced CP4 EPSPS protein was deduced from the coding region of the *cp4 epsps* gene present in MON 88017. Masses were calculated for each theoretical peptide and the raw mass data was compared to the calculated theoretical masses. Experimental masses (MH+) were assigned to peaks when three (or more) isotopically resolved ion peaks were observed in the raw mass data. Peaks were not assessed if there were less than three isotopically resolved peaks in the spectra, when peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned because of overlap with a stronger signal ±2 daltons from the mass analyzed. Known autocatalytic fragments from trypsin digestion were identified in the raw data.

B.1.h.3. Molecular Weight Determination using MALDI-TOF MS

Prior to analysis, the plant-produced CP4 EPSPS protein and NIST BSA reference protein were desalted using drop dialysis (Görisch, 1988). Briefly, 4μL of protein was placed on a Millipore 25 mm microdialysis disk (type VSWP, 0.025 μm pore size) and dialyzed for 60-120 minutes against HPLC-grade water. A portion of each protein sample (0.3 and 0.5 μL) was spotted on an analysis plate, mixed with 0.75 μL sinapinic acid solution, and air-dried. Mass spectral analysis of the plant-produced CP4 EPSPS

protein was performed using an Applied Biosystems Voyager DE-Pro Biospectrometry Workstation Matrix Assisted Laser Desorption and Ionization (MALDI) Time of Flight (TOF) instrument with the supplied Data Explorer software (version 4.0). Mass calibration of the instrument was performed using the desalted BSA reference protein. The mass of the plant-produced CP4 EPSPS protein was reported as an average of three separate mass spectral acquisitions. For comparison, the mass of the CP4 EPSPS protein was calculated from the expected amino acid sequence of the protein using the Protean module of DNAstar software (version 5.01).

B.1.i. Purity and Molecular Weight Determination using SDS-PAGE

An aliquot of the plant-produced CP4 EPSPS protein was diluted and mixed with loading buffer to a final concentration of 1× loading buffer. The sample was heated at ~102 °C for 4 min prior to loading onto a Tris-glycine buffered 4-20% gradient polyacrylamide gel. Three amounts (1, 2, and 3 μg) of total protein were loaded into three separate wells. Bio-Rad broad range molecular weight markers (1 μg each protein/lane) were included on the gel to estimate the molecular weight of the test substance. Electrophoresis was performed at a constant voltage of 125 V for 100 min. Proteins were fixed in the gel for 60 min in a solution containing 40% (v/v) methanol and 7% (v/v) glacial acetic acid, then stained for 2 h with Colloidal Brilliant Blue G stain (Sigma, St. Louis, MO). The gel was destained for 45 sec in a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, followed by 2 h in a solution containing 25% (v/v) methanol.

Analysis of the gel was performed using a Bio-Rad Laboratories GS-710 densitometer with the supplied Quantity One software, version 4.3.0. Molecular weight values of the marker proteins were supplied by the manufacturer and used to estimate the molecular weight of each observed band in the plant-produced CP4 EPSPS protein. All visible bands within each sample lane were quantified. Purity was estimated as the percent optical density of the ~45 kDa band relative to all bands detected in the lane. Molecular weight and purity were reported as an average of all three lanes containing the plant-produced CP4 EPSPS protein. The plant- and *E. coli*-produced proteins were considered to have equivalent molecular weights if the difference in the molecular weights was ≤ 5%.

B.1.j. Phosphate Release Assays

Prior to analysis, the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were diluted in a buffer solution containing 50 mM HEPES, pH 7.0. Enzymatic activity assays for both the plant- and the *E. coli*-produced CP4 EPSPS proteins were conducted in duplicate or triplicate and each replicate assay was subsequently analyzed in duplicate spectrophotometrically. This end-point type colorimetric assay measures the release of inorganic phosphate from one of the substrates, phosphoenolpyruvate (PEP), which is released by the action of the EPSPS enzyme in the presence of shikimate-3-phosphate (S-3-P). Reaction mixtures contained the EPSPS enzyme with 2 mM S-3-P and were initiated with 1 mM PEP (final concentration). The final reagent concentrations in the assay were 50 mM HEPES (pH

7.0), 0.1 mM ammonium molybdate and 5 mM potassium fluoride. Reactions were incubated for two minutes at 25 °C to allow for product formation. The reactions were quenched with malachite green reagent and fixed two minutes later with 33% (w/v) sodium citrate. The EPSPS-catalyzed release of inorganic phosphate from PEP was determined at a wavelength of 660 nm using a PowerWave X_i microplate reader, relative to a standard curve of inorganic phosphate treated with the malachite green reagent and 33% (w/v) sodium citrate. For EPSPS, one unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 μmole of inorganic phosphate from PEP per minute at 25 °C. Calculations of the specific activities were performed using Microsoft Excel 2000 version 9.0.4402 SR-1. Specific activity values were calculated based on the assay concentration of the CP4 EPSPS protein. The plant and *E. coli*-produced proteins were considered to have equivalent functional activity if their specific activities differed by ≤ 2 fold.

B.1.k. Glycosylation Analysis

Glycosylation analysis was performed to determine whether the plant-produced CP4 EPSPS protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the test substance, the *E. coli*-produced CP4 EPSPS reference standard (a negative control), and transferrin (a positive control) were each diluted and mixed with loading buffer to a final concentration of 1× loading buffer. The samples were heated at 99-101 °C for 4 min, and two amounts (0.5 and 1 μg) of each sample were loaded, along with Bio-Rad prestained broad range molecular weight markers (10 μL/lane), on a Tris-glycine buffered 4-20% gradient polyacrylamide gel. Electrophoresis was performed at a constant voltage of 170 V for 70 min. After electrophoresis proteins were electrotransferred to a 0.45 μm PVDF membrane for 90 min at a constant current of 300 mA.

Carbohydrate detection was performed directly on the PVDF membrane using the ECL glycoprotein detection system (Amersham Pharmacia). The PVDF membrane was gently shaken for 10 min in PBS and transferred to a solution of 100 mM sodium acetate containing 10 mM sodium metaperiodate. The membrane was incubated in the dark for 20 min in the oxidation reagent. The oxidation solution was removed from the membrane by two brief rinses with PBS followed by three sequential 10 min washes in PBS. The membrane was transferred to a solution of 100 mM sodium acetate containing 25 nM biotin hydrazide and incubated for 60 min. The biotin hydrazide solution was removed by rinsing the membrane twice in PBS followed by washing the membrane in PBS three times for 10 min each. The membrane was blocked overnight in a 4 °C refrigerator in the blocking agent supplied with the kit. The blocking solution was removed by rinsing the membrane twice in PBS followed by washing the membrane in PBS three times for 10 min each. The membrane was incubated with streptavidin-HRP conjugate (diluted 1:6000) in PBS for 30 min to detect carbohydrate moieties bound to biotin. Excess streptavidin-HRP was removed by rinsing and washing as previously described. Bands were visualized using the ECL detection system and the membrane was exposed (30 sec, 3 min, and 10 min) to Hyperfilm ECL high performance

chemiluminescence film. Films were developed using a Konica SRX-101A automated film processor.

B.2. Results

B.2.a. Western blot analysis to confirm the identity of the CP4 EPSPS protein

A protein band migrating at an approximate molecular weight of 45 kDa on SDS-PAGE gel was identified as the CP4 EPSPS protein by western blot analysis using a goat anti-CP4 EPSPS antibody (Figure B-1, Lanes 9-14). This serum previously was shown to be specific for the CP4 EPSPS protein and therefore was used to confirm the identity of the plant-produced CP4 EPSPS protein. One immunoreactive band migrating at approximately 45 kDa was observed in both the *E. coli*- and plant-produced CP4 EPSPS samples, confirming the identity of the CP4 EPSPS protein produced in MON 88017.

B.2.b. Molecular weight and purity of CP4 EPSPS protein

Molecular weight and purity of the plant-produced CP4 EPSPS protein were estimated using densitometric analysis of a Colloidal Brilliant Blue G stained SDS-polyacrylamide gel (Figure B-2). Out of three bands observed on the SDS-polyacrylamide gel, the 45 kDa band was identified as CP4 EPSPS protein by western blot analysis. The predicted molecular weight of the CP4 EPSPS protein, based on the deduced amino acid sequence, is 47.7 kDa. The apparent molecular weight of the plant-produced CP4 EPSPS protein, estimated by comparison to molecular weight markers on the SDS-polyacrylamide gel, was 45 kDa and the plant- and *E. coli*-produced proteins co-migrated on a Tris-glycine buffered 4-20% polyacrylamide gel (Figure B-2). The purity value for the plant-produced CP4 EPSPS protein calculated as the average of three separate loads was 45.5%.

The molecular weight of the plant-produced CP4 EPSPS protein was further confirmed using MALDI-TOF mass spectrometry. The average mass (MH+), determined from three separate spectral acquisitions, was 47,447.25 daltons. This value compares well with the predicted mass for CP4 EPSPS, 47,730 daltons.

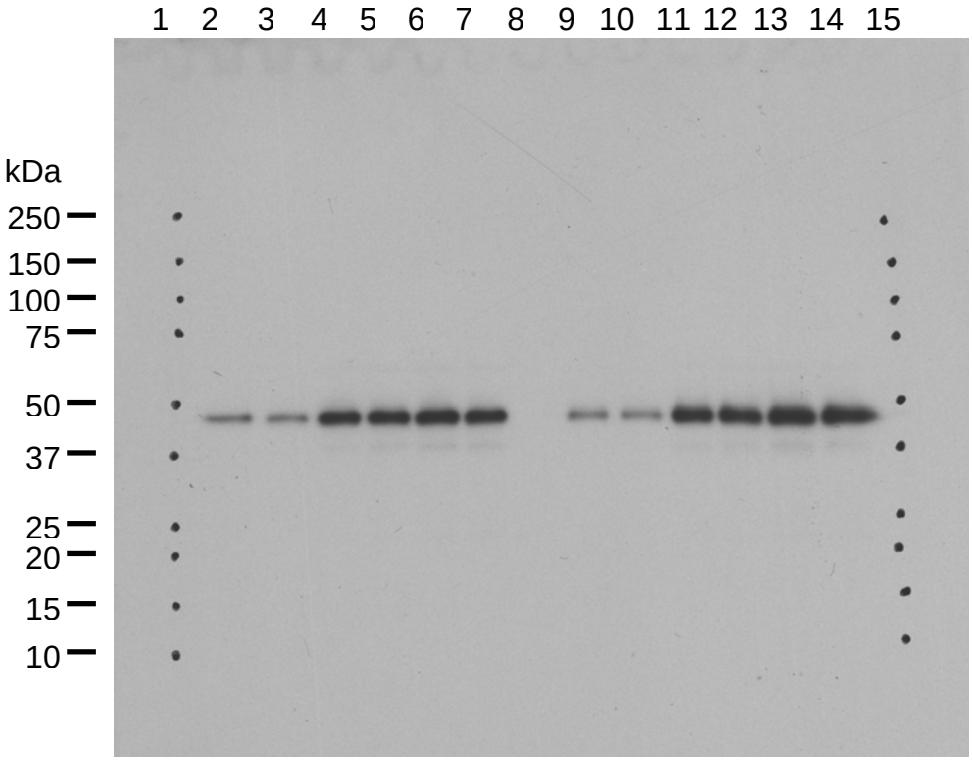


Figure B-1. Western Blot Analysis Demonstrating equivalence of CP4 EPSPS Proteins Produced in MON 88017 and *E. coli*

Samples of the plant- and *E. coli*-produced CP4 EPSPS proteins (1, 2, and 3 ng) were separated using SDS-PAGE and transferred to a PVDF membrane. The membrane was incubated with goat anti-CP4 EPSPS polyclonal antiserum to detect the presence of CP4 EPSPS protein. The dots (Lanes 1 and 15) represent the location of the molecular weight markers (MWM) on the membrane. A 10 sec exposure is shown.

Lane	Sample	Amount (ng)
1	Bio-Rad Precision Plus Dual Color MWM	---
2	*E. coli*-produced CP4 EPSPS	1
3	*E. coli*-produced CP4 EPSPS	1
4	*E. coli*-produced CP4 EPSPS	2
5	*E. coli*-produced CP4 EPSPS	2
6	*E. coli*-produced CP4 EPSPS	3
7	*E. coli*-produced CP4 EPSPS	3
8	loading buffer	---
9	plant-produced CP4 EPSPS	1
10	plant-produced CP4 EPSPS	1
11	plant-produced CP4 EPSPS	2
12	plant-produced CP4 EPSPS	2
13	plant-produced CP4 EPSPS	3
14	plant-produced CP4 EPSPS	3
15	Bio-Rad Precision Plus Dual Color MWM	---

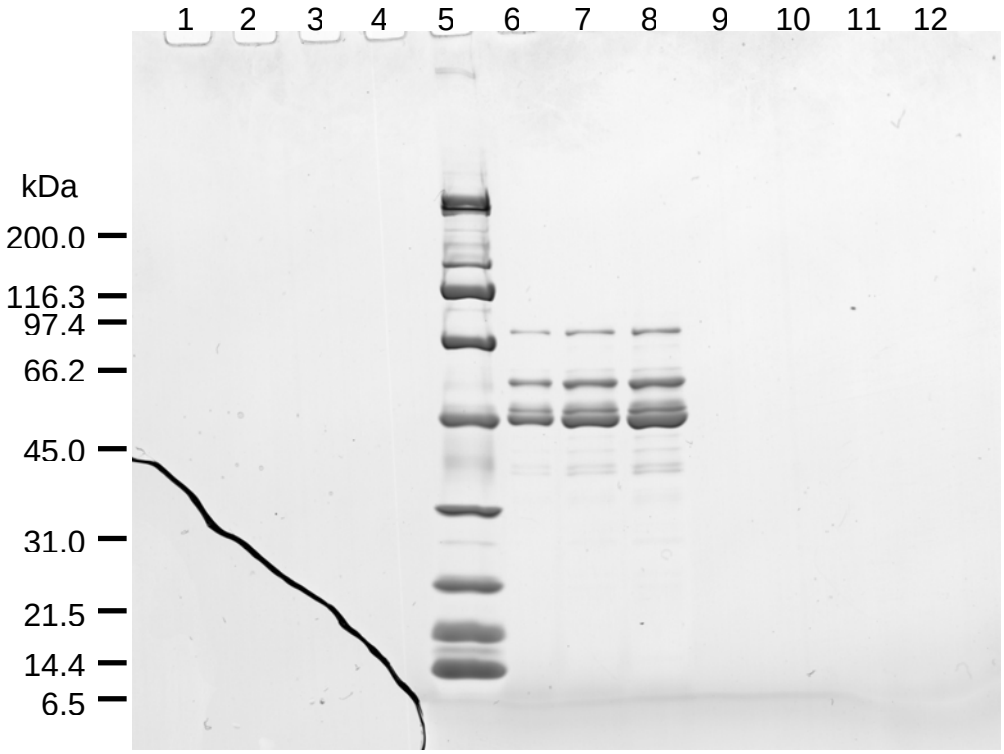


Figure B-2. Molecular Weight and Purity of the Plant-Produced CP4 EPSPS Protein Demonstrated by SDS-PAGE Analysis

1, 2 and 3 µg total protein of plant-produced CP4 EPSPS protein sample were separated by electrophoresis on a 4-20% gradient polyacrylamide gel under reducing and denaturing conditions. The stained gel was analyzed using densitometry to determine the purity and molecular weight of the CP4 EPSPS protein.

Lane	Sample	Amount (µg)
1	empty	---
2	empty	---
3	loading buffer	---
4	loading buffer	---
5	Bio-Rad Broad Range MWM	---
6	plant-produced CP4 EPSPS	1
7	plant-produced CP4 EPSPS	2
8	plant-produced CP4 EPSPS	3
9	loading buffer	---
10	loading buffer	---
11	empty	---
12	empty	---

B.2.c. MALDI-TOF MS tryptic map mass analysis

MALDI-TOF mass spectrometry analysis was performed on the CP4 EPSPS protein produced in MON 88017. The protein band identified as CP4 EPSPS was excised from the gel, chemically reduced, alkylated, digested with trypsin, and analyzed using MALDI-TOF MS. The ability to identify a protein using this method is dependent upon matching a sufficient number of observed tryptic peptide fragment masses with theoretical tryptic peptide fragment masses. With sufficient mass accuracy, four tryptic peptides may be sufficient to identify a protein (Jiménez et al., 1998). Peptides were considered to match when a difference in molecular weight of less than one dalton was found between the observed and theoretical fragment masses. For the 45kDa protein, a total of 22 observed peptide mass fragments matched the theoretical tryptic peptide mass map for the CP4 EPSPS protein.

The amino acid sequence of the plant-produced CP4 EPSPS protein was deduced from the coding region of the *cp4 epsps* gene present in MON 88017. A coverage map was generated using the identified masses from the MALDI-TOF MS tryptic mass analysis. Approximately 55.2% (251 of 455 amino acids) of the expected protein sequence was identified (Figure B-3). The predicted molecular weight of the full-length CP4 EPSPS protein, based on the deduced amino acid sequence, is 47.7 kDa. Using the above criteria, the 45kDa plant-produced protein band was identified as CP4 EPSPS protein.

```
  1   MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL
 51   LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA
101   TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD
151   RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR
201   DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF
251   PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED
301   VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL
351   RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT
401   HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS
451   DTKAA
```

Figure B-3. MALDI-TOF MS Coverage Map of the CP4 EPSPS Protein Sequence Produced in MON 88017

The amino acid sequence of the plant-produced CP4 EPSPS protein was deduced from the coding region of the *cp4 epsps* gene present in MON 88017. A coverage map was generated using the identified masses from the MALDI-TOF MS tryptic mass analysis. Approximately 55.2% (251 of 455 amino acids) of the expected protein sequence (indicated by the shaded area) was identified. The predicted molecular weight of the full-length CP4 EPSPS protein, based on the deduced amino acid sequence, is 47.7 kDa.

B.2.d. N-terminal sequence analysis

An SDS-PAGE analysis of the plant-produced CP4 EPSPS protein revealed three protein bands (migrating at approximately 45, 48, and 55 kDa, Figure B-2), each representing greater than 10% of the total protein in the plant-produced CP4 EPSPS sample. The identity of these bands was evaluated using N-terminal sequence analysis (Table B-1). The observed amino acid sequence for the 45 kDa protein band matched the expected N-terminal amino acid sequence of the CP4 EPSPS protein. Two sequences, both consistent with the N-terminus of the CP4 EPSPS protein were observed in the plant-produced CP4 EPSPS protein sample. The primary sequence originates at residue 2 (leucine) and matches the expected N-terminal amino acid sequence of the CP4 EPSPS protein from residues 2 through 16 (Table B-1). It is not uncommon for the initiator methionine to be removed from proteins in eukaryotic organisms by an endogenous methionine aminopeptidase (Arfin and Bradshaw, 1988). The secondary sequence starts at residue 4 (glycine) and matches the expected N-terminal amino acid sequence of the CP4 EPSPS protein from residues 4 through 18 (Table B-1). The loss of several N-terminal amino acid residues may be because of protease action when plant cells are homogenized. Despite the ragged N-terminus, the sequence data confirm the identity of the 45 kDa protein in the plant-produced sample as the CP4 EPSPS protein.

The N-terminal sequence of the 48 kDa protein band was obtained and the sequence did not match that of the CP4 EPSPS protein. The sequence obtained was used to search the non-redundant public database and the PIR Protein database but no matches were identified. Only five amino acid residues were obtained from the N-terminal sequence analysis of the 55 kDa protein and therefore no positive identification could be made for this protein.

Table B-1. N-Terminal Sequence of CP4 EPSPS Protein Produced in MON 88017

MW	Observed Sequence	Identity
45 kDa	Major Sequence LHGASSRPATARKS(S)	Residues 2-16 of CP4 EPSPS
	Secondary Sequence GASSRPATARKS(S)G(L)	Residues 4-18 of CP4 EPSPS
48 kDa	GGFKVTRISEGPVKX	Not Identified
55 kDa	(G)LIDGXXXXXXXXXX	Not Identified

Sequences obtained for each protein band were compared with the known sequence for CP4 EPSPS and/or against protein sequence databases. The ( ) denotes tenuous amino acid designations and Xs refer to undesignated amino acids. Amino acid residues 1-18 (using the IUPAC-IUB amino acid codes) of the CP4 EPSPS protein are MLHGASSRPATARKSSGL.

B.2.e. Functional activity

The specific activities of the plant- and *E. coli*-produced CP4 EPSPS proteins were estimated concurrently using a phosphate release assay. The specific activity of the plant-produced protein was 7.1 U/mg of CP4 EPSPS, and the specific activity of the *E. coli*-produced CP4 EPSPS protein was 6.0 U/mg. This confirmed that the functional activity of the CP4 EPSPS produced in MON 88017 is similar to that of the *E. coli*-produced CP4 EPSPS.

B.2.f. Glycosylation analysis

Many eukaryotic proteins are post-translationally modified with carbohydrate moieties (Rademacher et al., 1988). These carbohydrate moieties may be complex branched polysaccharide structures or simple monosaccharides. In contrast, prokaryotic organisms such as *E. coli* lack the necessary organelles and biochemical pathways required for protein glycosylation. To test if post-translational glycosylation of the plant-produced CP4 EPSPS protein occurred, this protein was analyzed for covalently bound carbohydrate moeities. The *E. coli*-produced CP4 EPSPS protein was analyzed concurrently as a negative control in this experiment. Transferrin protein was analyzed as a positive control. The positive control (transferrin) was clearly detected in a concentration-dependent manner at loadings of 0.5 and 1 μg/lane (Figure B-4, Lanes 2-3). No bands were observed in the *E. coli*- or plant-produced protein samples (Lanes 4-7). Several dots present on the blot were considered to be nonspecific background because of their form and location. Because no band was observed at the expected molecular weight for the plant- or *E. coli*-produced CP4 EPSPS proteins, it was concluded that the proteins were not glycosylated.

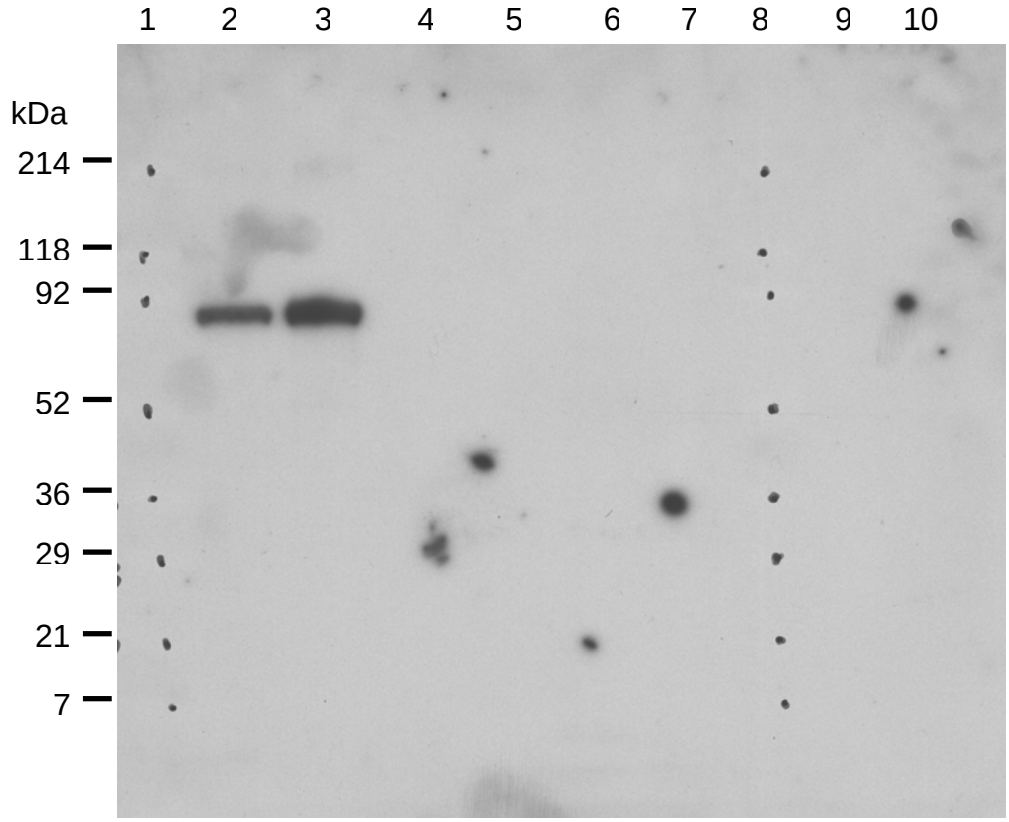


Figure B-4. Glycosylation Analysis of the CP4 EPSPS Protein Produced in MON 88017

Proteins were separated by SDS-PAGE under reducing and denaturing conditions and transferred to a PVDF membrane. The dots (Lanes 1 and 8) represent the location of the molecular weight markers (MWM) on the membrane. A three minute exposure is shown.

Lane	Sample	Amount (µg)
1	Bio-Rad Prestained Broad Range MWM	---
2	transferrin	0.5
3	transferrin	1.0
4	*E. coli*-produced CP4 EPSPS	0.5
5	*E. coli*-produced CP4 EPSPS	1.0
6	plant-produced CP4 EPSPS	0.5
7	plant-produced CP4 EPSPS	1.0
8	Bio-Rad Prestained Broad Range MWM	---
9	empty	---
10	empty	

Appendix C: Materials, Methods and Results of the Characterization of the Cry3Bb1 Protein

A panel of analytical tests was used to characterize the plant-produced Cry3Bb1 protein: (1) Western blot analysis and densitometry, (2) SDS-PAGE and densitometry, (3) MALDI-TOF mass spectrometry, (4) glycosylation analysis, and (5) a Cry3Bb1 bioactivity assay. The equivalence of the plant-produced Cry3Bb1 protein to the *E. coli*-produced Cry3Bb1 protein was evaluated by comparing their full-length molecular weights, immunoreactivity with anti-Cry3Bb1 antibodies, glycosylation status and functional activity. Section C.1 describes the materials and methods used and Section C.2 provides the results of the analytical tests.

C.1. Materials and Methods

C.1.a. Plant-Produced Cry3Bb1 Protein

The plant-produced Cry3Bb1 protein was isolated from MON 88017 grain. The identity of the MON 88017 corn grain sample was confirmed using event-specific PCR. The Cry3Bb1 protein was stored in a –80 °C freezer in a buffer solution containing 100 mM Tris-HCl, pH 8.0.

C.1.b. *E. coli*-Produced Cry3Bb1 Protein

The *E. coli*-produced Cry3Bb1 protein (lot 30-100002) was used as a reference standard to establish equivalence in select analyses. The Cry3Bb1 protein was stored in a –80 °C freezer in a buffer solution [50 mM sodium carbonate-bicarbonate, pH 10.1, 1 mM EDTA] at a total protein concentration of 1.2 mg/mL by amino acid analysis.

C.1.c. Assay Controls

Bovine serum albumin (BSA) protein was used to estimate the total protein concentration in the Bio-Rad protein assays. Protein molecular weight markers were used to calibrate SDS-polyacrylamide gels and verify protein transfer to PVDF membranes. A peptide mixture was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. Transferrin was used as a positive control in glycosylation analysis.

C.1.d. Protein Purification

The Cry3Bb1 protein was purified from extracts of ground defatted MON 88017 grain using a combination of anion exchange chromatography and affinity chromatography.

Cry3Bb1 protein was extracted from three batches (1-1.5 kg each) of defatted MON 88017 grain with either 50 mM Tris-HCl, pH 8.0 buffer or 50 mM sodium carbonate/bicarbonate, 1 mM EDTA, pH 10 buffer (buffer A) [1:10 (w/v) grain to buffer ratio]. The extracts were clarified by centrifugation and the supernatant concentrated 3-4

fold. The samples not extracted in buffer A were combined and dialyzed into buffer A. Samples were loaded onto a Q-sepharose Fast Flow anion exchange column that was pre-equilibrated with buffer A. The column was washed with 25% buffer B (50 mM sodium carbonate/bicarbonate, 0.5 M NaCl, 1 mM EDTA, pH 10 (buffer B). The Cry3Bb1 protein was eluted with 45% buffer B. The protein was concentrated and dialyzed into 50 mM Tris-HCl, pH 8.0. The dialyzed sample was loaded onto protein G agarose columns coupled with monoclonal Cry3Bb1 antibodies for affinity chromatography. The sample was recirculated over the antibody column. Cry3Bb1 protein was eluted from the column with 100 mM triethylamine, pH 11.2. Fractions were collected into 100 mM Tris-HCl, pH 8.0, to neutralize the solution. The fractions were concentrated and buffer exchanged into 100 mM Tris-HCl, pH 8.0. Fractions containing proteins similar in molecular weight to the Cry3Bb1 protein were pooled based on visual examination of Colloidal Brilliant Blue G stained SDS polyacrylamide gels.

C.1.e. Total Protein Concentration Determination

The total protein concentration of the plant-produced Cry3Bb1 protein was estimated using a Bio-Rad (Hercules, CA) protein concentration assay. BSA protein (concentrations ranging from 0.05 to 0.6 mg/mL) was used to create a standard curve. The Cry3Bb1 protein concentration was estimated by comparison to the linear standard curve. Data was collected using a Bio-Tek Instruments, Inc. Powerwave Xi microplate scanning spectrophotometer (Winooski, VT) employing the KC4 software version 2.6 revision 3. Readings were taken at a wavelength of 595 nm.

C.1.f. Western Blot Analysis

The method used was similar to that described in Appendix B.1.f. The detection reagents specific to this procedure were goat anti-Cry3Bb1 serum (lot 6844582) and anti-goat IgG, HRP conjugated antibody.

The percent difference in immunoreactivity between the plant- and *E. coli*-produced Cry3Bb1 proteins was calculated using Microsoft Excel 2000. The proteins were considered to have equivalent immunoreactivity if the average percent difference was ≤ 10%.

C.1.g. MALDI-TOF MS Tryptic Mass Map Analysis

The method used was similar to that described in Appendix B.1.h. Bands that corresponded to the sizes expected for full-length Cry3Bb1 protein (~77 kDa) and its major fragments (~66 and ~55 kDa) were excised, destained, reduced, alkylated, and subjected to an in-gel trypsin digest (Williams et al., 1997) followed by MALDI-TOF MS analysis.

C.1.h. Purity and Molecular Weight Determination using SDS-PAGE

The method used was similar to that described in Appendix B.1.i. The plant and *E. coli*-produced proteins were considered to have equivalent molecular weights if their molecular weights differed by ≤ 5%.

C.1.i. Insect Bioassays

In order to assess functional activity of Cry3Bb1 protein produced in MON 88017, the plant- and *E. coli*-produced Cry3Bb1 proteins were used to estimate the bioactivity (measured as an LC_{50} value) of the Cry3Bb1 proteins incorporated into diets fed to Colorado potato beetle (CPB) larvae, a susceptible insect. The plant and *E. coli*-produced proteins were considered to have equivalent functional activity if the difference in LC_{50} values was four-fold.

C.1.i.1. Samples

The test samples analyzed were the *E. coli*- and plant-produced Cry3Bb1 protein, lot numbers 30-100002 and 60-100024, respectively, suspended in buffer. The *E. coli*-produced-protein was suspended in a 100 mM Tris-HCl buffer, pH 8.0, and the plant produced Cry3Bb1 was suspended in a 50 mM sodium carbonate buffer, pH 10, with 1mM EDTA. Samples of each of the buffer types were received for use as control treatments. The test samples and buffers were stored in a -80° C freezer and a 5° C refrigerator, respectively.

C.1.i.2. Insects

Colorado potato beetle eggs were obtained from the New Jersey Department of Agriculture, West Trenton, NJ. Insect eggs were incubated at temperatures ranging from 15°C to 27°C depending on desired hatch times.

C.1.i.3. Bioassays

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, was used to measure activity of the plant- and *E. coli*-produced Cry3Bb1 protein samples. The bioassay was replicated two times on different days, each using a separate batch of insects. Each bioassay replicate consisted of a series of dilutions from each Cry3Bb1 sample yielding a dose series ranging from 0-3 μg Cry3Bb1 protein/ml diet plus a buffer control. Each bioassay also included a purified water control. The Cry3Bb1 protein doses were prepared by diluting the protein with purified water and incorporating the dilution into an agar-based Colorado potato beetle diet (Bio-Serv, Frenchtown, NJ). This dose series in diet was expected to elicit a response range from CPB larvae that would allow for an estimate of the LC_{50} of the Cry3Bb1 protein. The diet mixture was then dispensed in 0.5 ml aliquots into a 128 well tray (#BIO-BA-128, CD International, Pitman, NJ). Insect larvae were placed on these diets, approximately 16 insects per treatment, using a soft, fine, natural hair paintbrush. The infested wells were covered by a ventilated adhesive

cover (#BIO-CV-16, CD International) and the insects were allowed to feed for a period of approximately seven days in an environmental chamber programmed at 27°C, ambient relative humidity and a lighting regime of 14:10 light:dark. The number of survivors was recorded after the incubation period. Control treatments weights were recorded for quality control purposes only and were not used in the analysis.

C.1.i.4. Statistical analysis

The bioassay data analyzed consisted of the number of surviving CPB larvae for each dose. Statistical analysis was performed with Release 8.2 of the SAS statistical program running under Windows 2000 Professional (SAS Institute Inc., 1999-2001). A Probit analysis was performed to test the effect of the *E. coli*- and plant-produced Cry3Bb1 proteins on the observed mortality and to estimate the dose level required for 50% mortality (LC_{50}) for each replicate with the following model:

$$p_k = \mathrm{C} + (1 - \mathrm{C})\mathrm{F}(Dose_k + e_k)$$

p_k: Observed proportion of mortality under a given dose;
C: Natural (threshold) mortality rate;
F: Logistic cumulative distribution function;
$Dose_k$: Dose effect in the scale of base 10 logarithm;
e_k: Random residual effect.

An integrated Probit analysis was also performed to test the differences between two protein sources with the following model:

$$p_{ijk} = \mathrm{C} + (1 - \mathrm{C})\mathrm{F}(Treat_i + Rep_j + Dose_{ijk} + e_{ijk})$$

p_{ijk}: Observed proportion of mortality;
C: Natural (threshold) mortality rate;
F: Logistic cumulative distribution function;
$Treat_i$: Treatment effect;
Rep_j: Effect of the replicate;
$Dose_{ijk}$: Dose effect in the scale of base 10 logarithm;
e_{ijk}: Random residual effect.

The Fisher's Exact test was used to test for significant mortality in water and buffer control samples. Significance of tests was determined at the 0.05 level.

C.1.j. Glycosylation Analysis

Glycosylation analysis was performed to determine whether the plant-produced Cry3Bb1 protein was post-translationally modified with covalently bound carbohydrate moieties. The procedure used was similar to that described in Appendix B.1.k.

C.2. Results

The Cry3Bb1 protein was purified from the grain of MON 88017 and from *E. coli* cultures that express the Cry3Bb1 protein. Proteins of three molecular weights were identified as the full-length Cry3Bb1 protein and protease-cleaved products of Cry3Bb1 (Figure C-1). Cry3Bb1 protein (predicted molecular weight of 74.4 kDa) is processed in corn to produce peptides with apparent molecular weights of ~66 and ~55 kDa. The ~55 kDa peptide is thought to correspond to the *B.t*. tryptic core protein described in the literature (Schnepf et al., 1998; Caroll et al., 1997). Four immunoreactive bands migrating at approximately 77, 66, 55, and 46 kDa were observed in the sample purified from MON 88017 grain. The immunoreactive band of 46 kDa may represent a degradation product of Cry3Bb1 protein but was not considered for further analysis because it was smaller than the expected size of the tryptic core protein.

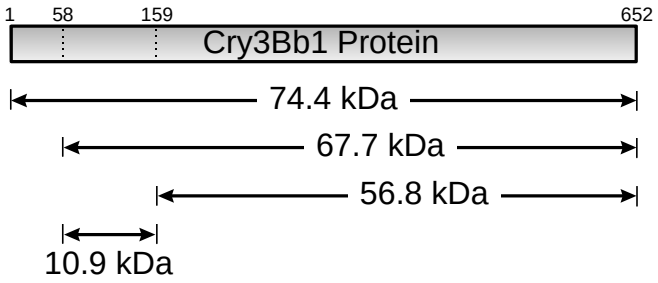


Figure C-1. Polypeptides Derived from the Full-Length Cry3Bb1 Protein

The amino acid positions are shown on top and correspond to residue numbers obtained from N-terminal sequence analysis of a homologous Cry3Bb1 protein. The predicted molecular weights for each polypeptide are shown.

Results obtained from the analyses conducted to characterize Cry3Bb1 protein produced in MON 88017 are discussed in the following sections.

C.2.a. Western blot analysis to confirm the identity of the Cry3Bb1 protein

Western blot analysis was performed using a goat anti-Cry3Bb1 serum. That serum was shown to be specific for Cry3Bb1 protein and was used to confirm the identity of the plant-produced Cry3Bb1 protein. Four immunoreactive bands migrating at approximately 77, 66, 55, and 46 kDa were observed (Figure C-2, lanes 9-14) in the plant-produced Cry3Bb1 sample. Full-length Cry3Bb1 protein with predicted molecular weight of 74.4 kDa is processed in corn to produce peptides with apparent molecular

weight ~66 kDa and ~ 55 kDa (Figure C-2). The immunoreactive band at 46 kDa may represent a degradation product of the Cry3Bb1 protein. This band was not analyzed because it was smaller than the expected size of the tryptic core determined for homologous Cry3Bb1 proteins.

The plant- and *E. coli*-produced Cry3Bb1 proteins were loaded in duplicate at three purity-corrected protein amounts: 1, 2, and 3 ng (Figure C-2). The percent difference in the immunoreactivity between the *E. coli*- and plant-produced Cry3Bb1 protein was calculated based on densitometric analysis of the bands for two different exposures (15 and 30 sec) of the western blot. The Cry3Bb1 antibody recognizes the plant-produced full-length protein and several smaller protein fragments that represent proteolytically cleaved forms of this protein. Bands greater than 55 kDa were included in the densitometric analysis of the plant-produced sample. The average difference n immunoreactivity between the plant- and *E. coli*-produced Cry3Bb1 proteins was 9%.

C.2.b. Molecular weight and purity of Cry3Bb1 protein

The molecular weight and purity of the plant-produced Cry3Bb1 protein were determined using densitometric analysis of a Colloidal Brilliant Blue G stained SDS-polyacrylamide gel (Figure C-3). The predicted molecular weight of the full-length Cry3Bb1 protein, based on the deduced amino acid sequence, is 74.6 kDa. The full-length Cry3Bb1 protein in the plant-produced sample had an estimated molecular weight of 77.2 kDa. The apparent molecular weight of the full-length *E. coli*-produced Cry3Bb1 protein, on the same gel, was estimated to be 77.7 kDa (Lane 7). The molecular weights of the additional protein fragments, previously identified as Cry3Bb1 protein (Section 1.2.a), were estimated to be 66.2 and 55.4 kDa. The purity of the plant-produced Cry3Bb1 protein was calculated as the sum of the three protein bands (77.2, 66.2, and 55.4 kDa) and the average from three separate loads (1, 2, and 3 μg) of total protein (Lanes 4-6). The average purity of the plant-produced Cry3Bb1 protein was 66.1%.

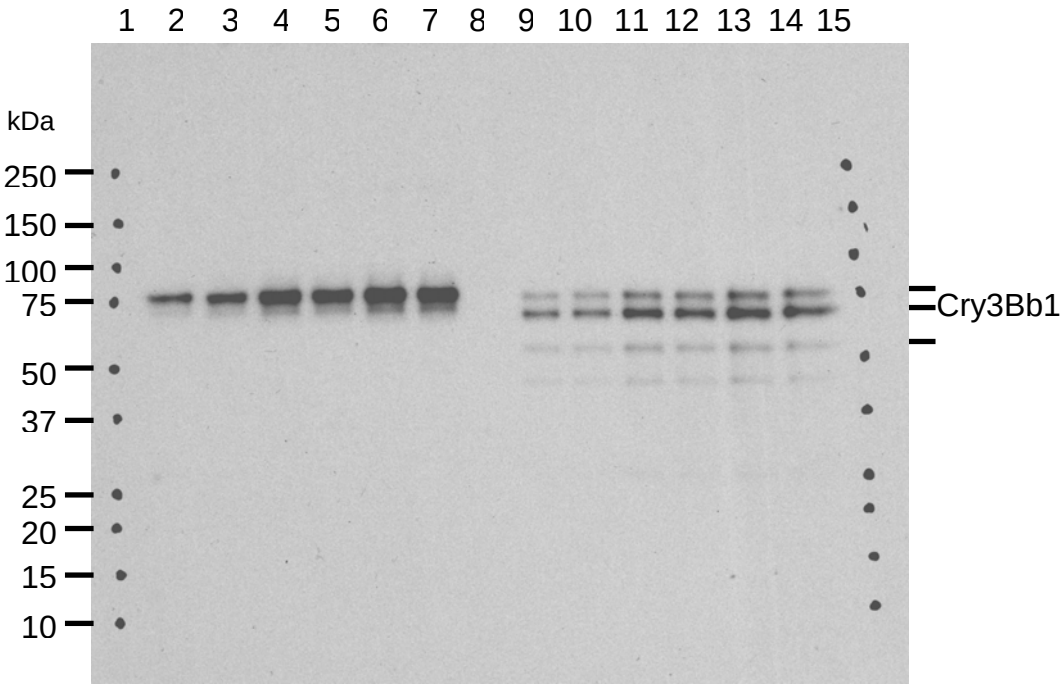


Figure C-2. Western Blot Analysis Demonstrating Equivalence of Plant- and *E.coli*-Produced Cry3Bb1 Proteins

Samples of the *E. coli*- and plant- produced Cry3Bb1 proteins (1, 2, and 3 ng) were separated using SDS-PAGE and transferred to a PVDF membrane. The membrane was incubated with goat anti-Cry3Bb1 antibodies to detect the presence of Cry3Bb1 protein. The dots (Lanes 1 and 15) represent the location of the molecular weight markers (MWM) on the membrane. A 15 sec exposure is shown.

Lane	Sample	Amount (ng)
1	Bio-Rad Precision Plus Dual Color MWM	---
2	*E. coli*-produced Cry3Bb1 protein	1
3	*E. coli*-produced Cry3Bb1 protein	1
4	*E. coli*-produced Cry3Bb1 protein	2
5	*E. coli*-produced Cry3Bb1 protein	2
6	*E. coli*-produced Cry3Bb1 protein	3
7	*E. coli*-produced Cry3Bb1 protein	3
8	2× loading buffer	---
9	plant-produced Cry3Bb1 protein	1
10	plant-produced Cry3Bb1 protein	1
11	plant-produced Cry3Bb1 protein	2
12	plant-produced Cry3Bb1 protein	2
13	plant-produced Cry3Bb1 protein	3
14	plant-produced Cry3Bb1 protein	3
15	Bio-Rad Precision Plus Dual Color MWM	---

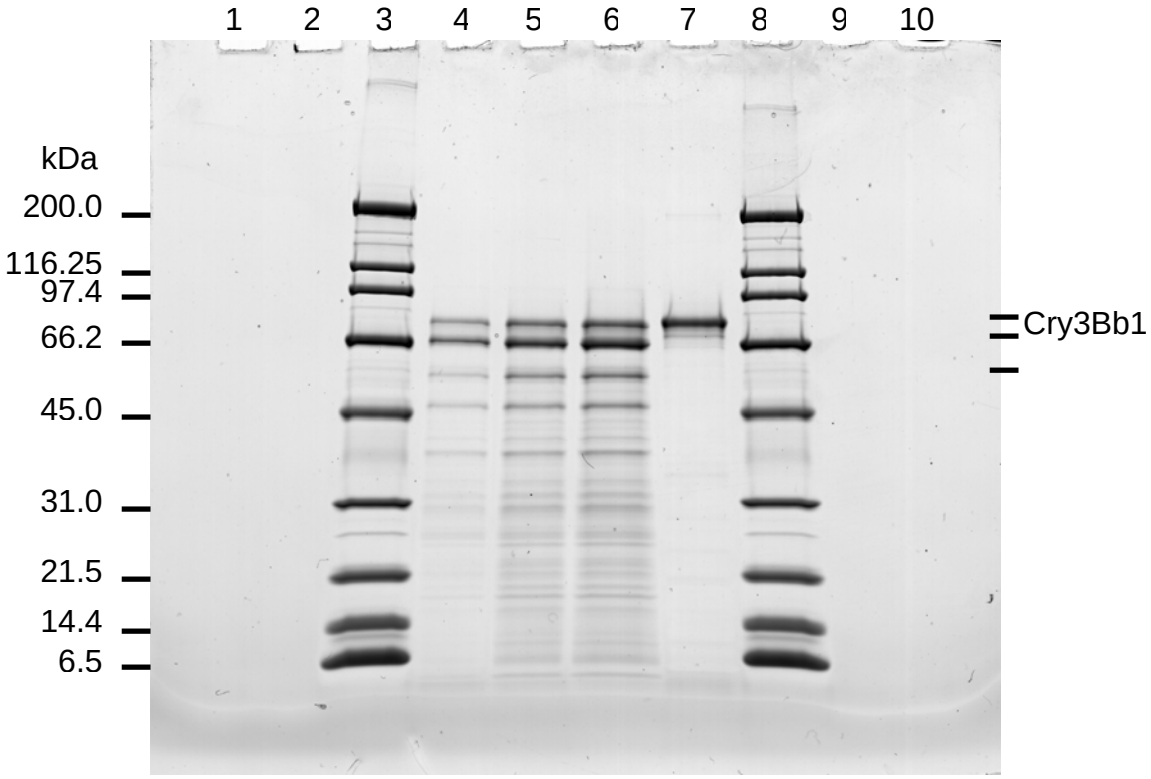


Figure C-3. Molecular Weight and Purity of the Cry3Bb1 Protein Demonstrated by SDS-PAGE Analysis

1, 2, and 3 µg of total protein from the plant-produced Cry3Bb1 protein sample and 1 µg of total proten from the *E. coli*-produced Cry3Bb1 protein sample were separated by electrophoresis on a 4-20% gradient polyacrylamide gel under reducing and denaturing conditions. The stained gel was analyzed using densitometry to determine the purity and molecular weight of the Cry3Bb1 protein.

Lane	Sample	Amount (µg)
1	empty	---
2	empty	---
3	Bio-Rad Broad Range MWM	---
4	plant-produced Cry3Bb1 protein	1
5	plant-produced Cry3Bb1 protein	2
6	plant-produced Cry3Bb1 protein	3
7	*E. coli*-produced Cry3Bb1 protein	1
8	Bio-Rad Broad Range MWM	---
9	empty	---
10	empty	---

C.2.c. MALDI-TOF MS tryptic mass map analysis

The identity of the plant-produced Cry3Bb1 protein was also assessed using MALDI-TOF mass spectrometry. The ability to identify a protein using this method depends upon matching a sufficient number of observed to expected (theoretical) mass fragments. With sufficient mass accuracy, four tryptic peptides may be enough to identify a protein (Jiménez et al., 1998). Peptides were considered to match when a difference in molecular weight of less than one dalton was found between the observed and theoretical fragment mass.

The identity of each of the three protein bands (~77, ~66 and ~55 kDa) observed in western blot analysis (Figure C-1) was confirmed. For the 77 kDa protein, 20 observed peptide mass fragments were matched to the theoretical tryptic peptide mass map for the fullp-length Cry3Bb1 protein. For the 66 kDa protein fragment, 20 observed peptide mass fragments were matched to the theoretical tryptic peptide mass map for the Cry3Bb1 protein. For the 55 kDa protein fragment, 15 observed peptide mass fragments were matched to the theoretical tryptic peptide mass map for the Cry3Bb1 protein. The identified masses were used to assemble a combined coverage map (Figure C-4) for the three bands identified as Cry3Bb1 protein in the western blot analysis. In total, 44% (287 of 653 amino acids) of the expected protein sequence was identified.

```
  1  MANPNNRSEH DTIKVTPNSE LQTNHNQYPL ADNPNSTLEE LNYKEFLRMT
 51  EDSSTEVLDN STVKDAVGTG ISVVGQILGV VGVPFAGALT SFYQSFLNTI
101  WPSDADPWKA FMAQVEVLID KKIEEYAKSK ALAELQGLQN NFEDYVNALN
151  SWKKTPLSLR SKRSQDRIRE LFSQAESHFR NSMPSFAVSK FEVLFLPTYA
201  QAANTHLLLL KDAQVFGEEW GYSSEDVAEF YRRQLKLTQQ YTDHCVNWYN
251  VGLNGLRGST YDAWVKFNRF RREMTLTVLD LIVLFPFYDI RLYSKGVKTE
301  LTRDIFTDPI FLLTTLQKYG PTFLSIENSI RKPHLFDYLQ GIEFHTRLRP
351  GYFGKDSFNY WSGNYVETRP SIGSSKTITS PFYGDKSTEP VQKLSFDGQK
401  VYRTIANTDV AAWPNGKVYL GVTKVDFSQY DDQKNETSTQ TYDSKRNNGH
451  VSAQDSIDQL PPETTDEPLE KAYSHQLNYA ECFLMQDRRG TIPFFTWTHR
501  SVDFFNTIDA EKITQLPVVK AYALSSGASI IEGPGFTGGN LLFLKESSNS
551  IAKFKVTLNS AALLQRYRVR IRYASTTNLR LFVQNSNNDF LVIYINKTMN
601  KDDDLTYQTF DLATTNSNMG FSGDKNELII GAESFVSNEK IYIDKIEFIP
651  VQL
```

Figure C-4. MALDI-TOF MS Coverage Map of the Cry3Bb1 Protein Sequence as Produced in MON 88017

Shaded regions correspond to tryptic peptide masses that were identified from the 77, 66, and 55 kDa bands, analyzed separately, using MALDI-TOF MS. The gray shaded sequence corresponds to the region identified from the 77 kDa band. The black shaded sequence corresponds to additional sequence identified from the 66 kDa band. No additional amino acids were identified from the 55 kDa band that were not observed in the 77 or 66 kDa bands.

C.2.d. Functional activity

The biological activity of *E. coli*- and plant-produced Cry3Bb1 proteins was estimated in a diet-incorporated bioassay. *E. coli*- and plant-produced Cry3Bb1 proteins were incorporated into a diet and fed to Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, larvae, a susceptible insect. Biological activity was measured as an LC_{50}, which is defined as the estimated lethal concentration of protein (μg/ml diet) required to kill 50% of the CPB larvae. The LC_{50} values for CPB when fed the plant- and *E. coli*-produced Cry3Bb1 proteins were 0.414 μg Cry3Bb1 protein/mL diet and 0.554 μg Cry3Bb1 protein/mL diet, respectively. Statistical analysis demonstrated that the two LC_{50} values were not different ($p>0.05$), leading to the conclusion that the *E. coli*- and plant-produced Cry3Bb1 proteins are functionally equivalent.

C.2.d. Glycosylation analysis

Many eukaryotic proteins are post-translationally modified with carbohydrate moieties (Rademacher et al., 1988). These carbohydrate moieties may be complex branched polysaccharide structures or simple monosaccharides. In contrast, prokaryotic organisms such as *E. coli* lack the necessary organelles and biochemical pathways required for protein glycosylation. To test if post-translational glycosylation of the plant-produced Cry3Bb1 protein occurred, this protein was analyzed for covalently bound carbohydrate moieties (Figure C-5). The *E. coli*-produced Cry3Bb1 protein and transferrin protein were analyzed concurrently as negative and positive controls, respectively. The positive control (transferrin) was clearly detected in a concentration-dependent manner at loadings of 0.25 and 0.5 μg/lane (Lanes 2-3). A band at ~25 kDa was observed in the *E. coli*-produced protein sample (Lanes 4-5) and no band was observed in the plant-produced Cry3Bb1 sample (Lanes 6-7). The band observed in the *E. coli*-produced Cry3Bb1 sample was at a lower molecular weight than the Cry3Bb1 protein. This band likely represents a naturally biotinylated protein that co-purified with the *E. coli*-produced Cry3Bb1 protein (Choi-Rhee et al., 2003), because it would bind to the streptavidin-HRP conjugate and be detected by the ECL system. No band was observed at the expected molecular weights for the Cry3Bb1 protein; therefore, it was concluded that it was not glycosylated.

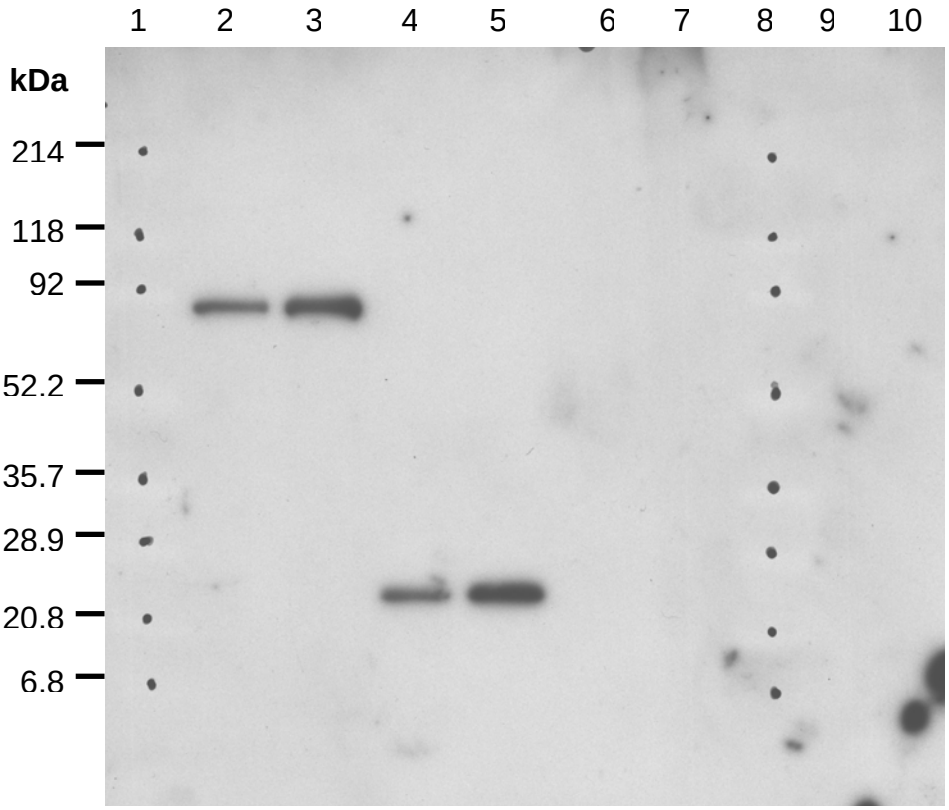


Figure C-5. Glycosylation Analysis of Cry3Bb1 Protein Produced in MON 88017

Proteins were separated using SDS-PAGE under reducing and denaturing conditions and transferred to a PVDF membrane. The dots in lanes 1 and 8 represent the location of the molecular weight markers (MWM) on the membrane. A 10 minutes exposure is shown.

Lane	Sample	Amount (µg)
1	Bio-Rad Prestained Broad Range MWM	---
2	transferrin	0.25
3	transferrin	0.5
4	*E. coli*-produced Cry3Bb1 protein	0.25
5	*E. coli*-produced Cry3Bb1 protein	0.5
6	plant-produced Cry3Bb1 protein	0.25
7	plant-produced Cry3Bb1 protein	0.5
8	Bio-Rad Prestained Broad Range MWM	---
9	empty	---
10	empty	

Appendix D. Materials and Methods for the Estimation of CP4 EPSPS and Cry3Bb1 Proteins

D.1. Test, Control, and Reference Substances

D.1.a. Test Substance

The test substance was MON 88017. The tissue types analyzed are described in Section D.2.b.

D.1.b. Control Substance

The control substance was the conventional corn hybrid H1200902 with genetic background similar to MON 80017. The control tissue types are described in Section D.2.b.

D.1.c. Characterization of Test and Control Substances

The identity of the test and control substances was confirmed by verifying the chain-of-custody documentation prior to analysis. To further confirm the identity of the test and control substances, event-specific polymerase chain reaction (PCR) analyses were conducted on seed and grain samples. Identity of grain samples harvested from the field was verified by PCR and the verification of identity was referenced back to the starting seed lot numbers.

D.1.d. Reference Substances

The *E. coli*-produced CP4 EPSPS protein standard (lot # 20-100015) was used as the reference substance for analysis of CP4 EPSPS protein levels. The purity-corrected total protein concentration of the purified standard was 3.7 mg/ml by amino acid composition analysis. The purity was 97% as determined by SDS-PAGE and densitometric analysis.

The *E. coli*-produced Cry3Bb1 protein standard (lot # 30-100002) was used as the reference substance for the analysis of Cry3Bb1 protein levels. The purity-corrected total protein concentration of the purified standard was 1.2 mg/ml by amino acid composition analysis. The purity was 98% as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis.

D.2. Generation of Plant Samples

D.2.a. Summary of Field Design

A production was initiated during the 2002 field season to generate test and control substances. The field sites were located within the major corn-growing region of the U.S. (Benton County, IA; Stark County, IL; and York County, NE) and provided a variety of

environmental conditions. At each site, three replicated plots of MON 88017 and control hybrid H1200902 were planted using a randomized complete block field design. Overseason leaf (OSL), overseason whole plant (OSWP), overseason root (OSR), pollen, silk, forage, forage root, grain, stover, and senescent root tissues were collected from each replicated plot at all field sites. Throughout the field production, sample identity was maintained by using unique sample identifiers and proper chain-of-custody documentation. Upon collection, all tissue samples were placed in uniquely labeled bags or containers. All tissue samples, except grain, were stored and shipped on dry ice to the Monsanto Company processing facility in St. Louis, Missouri. Grain samples were stored and shipped at ambient temperature.

D.2.b. Description of the collected tissues

Grain

All hand-pollinated ears in each test and control plot were collected at the R6 growth stage (physiological maturity). The ears were shucked and dried to moisture content of 10-15%.

Forage

Forage is defined as the entire plant (leaves, ears, tassels, and stalk) with the root ball removed. Two whole, self-pollinated plants at the early dent growth stage (R4 - R6) were collected from each test and control plot. The forage tissue samples were pooled from each plot during collection.

Pollen

Approximately 5 g of pollen was collected nonsystematically from each test and control replicated plot at each site at the R1 plant growth stage. Any debris in the pollen sample was removed using a fine mesh sieve.

Silk

Silks were collected nonsystematically from five primary ears of plants from each test and control plot at each site. The samples were collected at the time of hand pollination (the R1 plant growth stage). The silk tissue samples were pooled from each plot during collection.

Stover

Stover is defined as the above ground portion of the plant remaining after grain harvest. Two stover samples were collected from each test and control plot, after the harvest of self-pollinated ears. The stover samples were pooled from each plot during collection.

Overseason Leaf

The youngest immature whorl leaf (two to four inches) samples were collected from 15 plants from each of the test and control plots. The first OSL samples (OSL-1) were collected at the V2 - V3 growth stage; OSL-2 samples were collected at the V5 stage; OSL-3 samples were collected at the V8 stage; and OSL-4 samples were collected at the V11 - V17 stage. The leaves corresponding to each growth stage were pooled from each plot during collection.

Overseason Whole Plant

Two whole plants were collected from each of the test and control plots. An OSWP sample consists of shoot tissue (above-ground portion of the plant including leaves, tassels, ears, etc.). The two whole plant samples were pooled from each plot during collection. At each field site, the OSWP tissue samples were collected to coincide with the collection of OSL-1 to OSL-4 and OSR-1 to OSR-4 samples.

Overseason Root

The OSR samples were the below ground root mass that had been cut from the corresponding OSWP sample. The two root samples were pooled from each plot during collection. At each field site, the OSR tissue samples were collected to coincide with the collection of OSL-1 to OSL-4 and OSWP-1 to OSWP-4 samples.

Forage Root

Forage root is defined as the root ball removed from the plant sampled for forage. Forage root tissue samples were collected at the early dent growth stage (R4 - R6) from each test and control plot. Collection of the forage root tissue was similar to that used for the overseason root tissue. The forage root tissue samples were pooled from two plants from each plot during collection.

Senescent Root

Senescent root is the below ground root ball removed from plants sampled for stover. Two senescent root samples were collected from each test and control plot and corresponded to the plants that had been collected for stover. Collection of the senescent root tissue was similar to that used for the overseason root tissue. The senescent root tissue samples were pooled from each plot during collection.

D.3. Tissue Processing and Protein Extraction Methods

D.3.a. Processing

All tissue samples produced at the field sites were shipped to Monsanto Company's processing facility. During the processing step, dry ice was combined with the samples

(except pollen) and then vertical cutters or mixers were used to thoroughly grind and mix the tissues. Processed tissue samples were stored in a –80°C freezer until needed.

D.3.b. Extraction

The Cry3Bb1 and CP4 EPSPS proteins were extracted from corn tissues using standard buffer extraction procedures. All processed tissues were kept on dry ice during extract preparation. All tissues were extracted using a Harbil mixer and insoluble material was removed from the extracts by using a Serum Filter System (Fisher Scientific, Pittsburgh, PA). The extracts were aliquoted and stored in a –80°C freezer until ELISA analyses.

D.4. ELISA Reagents and Methods

D.4.a. CP4 EPSPS Antibodies

Mouse monoclonal antibody clone 39B6 (IgG2a isotype, kappa light chain; lot # 6199732) specific for the CP4 EPSPS protein was purified from mouse ascites fluid using Protein-A Sepharose affinity chromatography.

The concentration of the purified IgG was determined to be 3.2 mg/ml by spectrophotometric methods. Production of the 39B6 monoclonal antibody was performed by TSD Bioservices, Inc. (Newark, DE). The purified antibody was stored in a buffer (pH 7.2) containing 0.02 M $Na_2HPO_4 \cdot 7H_2O$, 0.15 M NaCl, and 15 ppm ProClin 300 (Sigma, St. Louis, MO). The detection reagent was goat anti-CP4 EPSPS antibody (produced by Sigma, St. Louis, MO) conjugated to horseradish peroxidase (HRP).

D.4.b. CP4 EPSPS ELISA Method

The CP4 EPSPS ELISA was performed using an automated robotic workstation. Mouse anti-CP4 EPSPS antibody was diluted in coating buffer containing 0.15 M NaCl to a final concentration of 1.0 µg/ml, and immobilized onto 96-well microtiter plates followed by incubation in a 4°C refrigerator for $\geq$8 h. Plates were washed in 1X PBS, CP4 EPSPS protein standard or sample extract was then added (100 µl/well), and plates were incubated at 37°C. Forage sample extracts were diluted with a 1X PBST with 0.1% (w/v) BSA solution and grain samples were diluted with a solution containing 0.1 M tris, 0.1 M $Na_2B_4O_7 \cdot 10H_2O$, 0.01 M $MgCl_2$, 0.05% (v/v) Tween-20 at pH 7.8, and 0.2% (w/v) L-ascorbic acid (TBA). Plates were washed as before followed by the addition of 100 µl per well of anti-CP4 EPSPS peroxidase conjugate and incubated at 37°C. Plates were developed by adding 100 µl per well of 3,3',5,5'- tetramethylbenzidine (TMB, Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantitation of CP4 EPSPS protein levels was accomplished by interpolation from a CP4 EPSPS protein standard curve that ranged in concentration from 0.456 - 14.6 ng/ml.

The assay was validated by the use of quality control samples. The positive quality control sample was prepared from tissue that contained the CP4 EPSPS protein. The

negative quality control sample was prepared from conventional tissue that did not contain the *cp4 epsps* coding sequence and therefore does not produce the CP4 EPSPS protein. Extracts of the positive and negative QC samples were analyzed on every plate in triplicate wells. All positive QC samples fell within the range established during method validation and all negative QC samples were less than the assay LOQ, as expected.

D.4.c. Cry3Bb1 Antibodies

Goat polyclonal antibody (lot # 7107417) specific for the Cry3Bb1 protein was purified using Protein-G Agarose affinity chromatography. The concentration of the purified IgG was determined to be 5.16 mg/ml by spectrophotometric methods. The purified antibody was stored in a phosphate buffered saline (PBS) buffer (pH 7.4) containing 0.001 M KH_2PO_4, 0.01 M $Na_2HPO_4 \cdot 7H_2O$, 0.137 M NaCl, and 0.0027 M KCl, with 0.02% NaN_3 (w/v) added as a preservative.

The purified antibody (lot # 7107417) was coupled with biotin (Sigma, St. Louis, MO) according to the manufacturer's instructions and assigned lot # 7107462. The detection reagent was NeutrAvidin (Pierce, Rockford, IL) conjugated to HRP.

D.4.d. Cry3Bb1 ELISA Method

The Cry3Bb1 ELISA was performed using an automated robotic workstation. Goat anti-Cry3Bb1 antibody was diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) and immobilized onto 96-well microtiter plates at a concentration of 5.0 µg/ml followed by incubation in a 4°C refrigerator for $\geq$12 h. Plates were washed with 1X PBST with 0.05% (v/v) Tween-20 (1X PBST), blocked with the addition of 150 µl per well of 1X PBS with 0.25% (w/v) casein buffer and incubated at 37°C. Plates were washed as before followed by the addition of 100 µl per well of the Cry3Bb1 protein standard or sample in 1X PBST/0.1% BSA and incubated at 37°C. The procedure was completed after separate incubations with the addition of 100 µl per well of biotinylated goat anti-Cry3Bb1 antibody and NeutrAvidin-HRP. Plates were developed by adding 100 µl per well of the HRP substrate TMB. The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantitation of Cry3Bb1 protein levels was accomplished by interpolation from a Cry3Bb1 protein standard curve that ranged in concentration from 0.35 - 11.2 ng/ml.

The assay was validated by the use of quality control samples. The positive quality control sample was prepared from tissue that contained the Cry3Bb1 protein. The negative quality control sample was prepared from conventional tissue that did not contain the *cry3Bb1* coding sequence and therefore does not produce the Cry3Bb1 protein. Extracts of the positive and negative QC samples were analyzed on every plate in triplicate wells. All positive QC samples fell within the range established during method validation and all negative QC samples were less than the assay LOQ, as expected.

Appendix E: Materials, Methods and Individual Site Results for Seed Dormancy and Germination

E.1. Materials And Methods

E.1.a. Facility Description

Personnel at a certified seed-testing laboratory conducted the seed germination analysis. The facility was qualified to conduct seed germination tests consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association (AOSA, 1998; AOSA, 2000). These testing guidelines provided an appropriate method to determine seed dormancy and germination characteristics. The laboratory was fully equipped with the appropriate equipment and trained personnel able to establish, monitor, and evaluate seed germination tests. The necessary equipment included temperature-regulated growth chambers able to maintain approximate constant temperatures from 5 to 30°C. These growth chambers were equipped with temperature monitoring and recording devices.

E.1.b. Test, Control, and Reference Starting Materials

Starting seed materials were produced by Monsanto Company at two production locations, Puerto Rico and Hawaii, in 2002. Seed materials included the test material, the control material, and three reference materials from each production location.

Test Material

The test material was MON 88017 with a genetic backgound of LH59 x LH198$BC3F_3$(+). Hemizygous MON 88017 corn plants with a LH198 background (BC3F2) were self-pollinated to create BC3F3 plants homozygous for MON 88017 and subsequently crossed to LH59 to make the test material.

Control Material

The control material was a conventional corn hybrid LH59 x LH198. The control material was tested to provide dormancy and germination characteristic values to which the test material could be compared.

Reference Materials

The reference materials were conventional corn hybrids HC33 x LH185, LH176 x HC33, LH59 x HC33 (Puerto Rico only), and HC33 x LH59 (Hawaii only). All were developed from commercially available elite inbreds. The reference materials provided a range of background values for dormancy and germination characteristics common to corn.

The identities of the test, control, and reference starting seed materials were verified by the presence or absence of the transgenes by MON 88017-specific polymerase chain reaction (PCR) analyses.

E.1.c. Experimental Methods

Temperature-controlled growth chambers with rolled towel tests to measure dormancy and germination characteristics were used. Four replicates of MON 88017, the control, and three reference hybrids, each grown at two production locations, were tested in seven temperature regimes ranging from 5 to 30ºC.

Each of the seven growth chambers were maintained dark under one of the following temperature regimes:

- Constant target temperature of approximately 5, 10, 20, or 30°C
- Alternating target temperatures of approximately 5/20, 10/20, or 20/30°C.

In the alternating temperature regimes, the lower temperature was maintained for 16 hours and the higher temperature for eight hours. Rolled germination towels containing exactly 100 seed of each test, control, and reference material were prepared according to standards established by AOSA (1998).

Each rolled germination towel was checked during the evaluation for germinated (normal and abnormal), dead, firm swollen (viable and nonviable), and hard (viable and nonviable, if any) seed using AOSA guidelines (AOSA, 1998). Nonviable firm swollen and hard seed were considered to be dead seed in the statistical analysis; thus, these categories were not reported. The definitions of each category were:

- Normal germinated seed exhibited normal development of the root and shoot with a shoot length of at least 0.5 inches (approximately 13 cm).
- Abnormal germinated seed lacked a well-developed root or shoot, or possessed a hollow coleoptile, or exhibited mechanical damage.
- Dead seed had visibly deteriorated and had become soft to the touch.
- Firm swollen seed had visibly swollen (imbibed water) and were firm to the touch.
- Hard seed did not imbibe water and remained hard to the touch.

Numbers of seed within each category for each temperature regime and replication were made on the 5th, 7th, and 12th days after experimental phase initiation.

- On the 5th day, normal germinated seed were counted and removed.
- On the 7th day, normal germinated seed, abnormal germinated seed, and dead seed were counted and removed. Firm swollen and hard seed (if any) were counted but left on the towel.
- On the 12th day, normal germinated seed, abnormal germinated seed, and dead seed were counted and removed. Remaining firm swollen and hard seed (if any)

were subjected to a tetrazolium test for evaluation of viability following AOSA guidelines (AOSA, 2000). Numbers of viable firm swollen, nonviable firm swollen, viable hard, and nonviable hard seed were recorded upon completion of the tetrazolium test. Any nonviable firm swollen or hard seed were added to the dead category prior to statistical analysis; thus, these categories were not reported.

E.1.d. Statistical Analysis

Variance analysis was conducted according to a complete random design using Statistical Analysis Software (SAS Version 8.2, SAS Institute, Inc. 1999-2001). Evaluation characteristics analyzed were percent normal germinated seed, percent viable hard seed, percent abnormal germinated seed, percent viable firm swollen seed, and percent dead seed. Nonviable hard and firm swollen seed were added to the dead seed prior to calculating percent dead seed. The test, control, and reference materials were tested independently for each combination of production location, temperature regime, and germination characteristic. No comparisons were made between temperature regimes or between production locations. Differences detected were statistically significant at $p \leq 0.05$.

E.2. Results

E.2.a. Percent Normal Germinated Seed

For the seed produced in Puerto Rico and Hawaii, no differences were detected in percent normal germinated seed between MON 88017 and the control in all temperature regimes except for the Hawaiian seed at 10/20ºC (Tables E-1 and E-2). In the 10/20ºC regime, the test seed from Hawaii had a higher percentage of normal germinated seed compared to the control (92.9 vs. 84.3%, respectively). The mean percent normal germinated seed values for MON 88017 were all within the range of values of the reference materials in all temperature regimes from both production locations.

E.2.b. Percent Hard Seed

No viable hard seed were observed for MON 88017, the control, or any of the references produced at either location (Tables E-1 and E-2). The lack of hard seed, a mechanism of seed dormancy, in MON 88017 corn indicates that the genetic modification process, the presence of the coding sequence, or the gene products did not alter dormancy mechanisms in the seed. Thus, it is concluded that there was no change in the pest potential of MON 88017 corn from increased dormancy through hard seed.

E.2.c. Percent Abnormal Germinated Seed

For the seed produced in Puerto Rico and Hawaii, no differences were detected in percent abnormal germinated seed between MON 88017 and the control in all temperature regimes, except for the seed at 10ºC from both production locations (Tables E-1 and E-2).

In the 10ºC temperature regime, MON 88017 corn seed from the Puerto Rico site had a lower percentage of abnormal germinated seed than the control (88.0 vs. 97.8%). In contrast, MON 88017 from Hawaii had a greater percentage of abnormal germinated seed than the control (91.5 vs. 82.5%). The high level of percent abnormal germinated seed at this temperature across all seed types was not unexpected, as 10ºC is below the optimum germination temperature for corn. The temperature was warm enough to promote germination, but not warm enough for proper seedling development. The mean percent abnormal germinated seed values for MON 88017 were within the range of values of the reference materials in all temperature regimes and from each production location except in the 10ºC temperature regime of the Hawaii production, which was slightly lower (91.5 vs. 93-100%).

E.2.d. Percent Viable Firm Swollen Seed

There were five differences in percent viable firm swollen seed detected between MON 88017 and the control (Tables E-1 and E-2). MON 88017 from the Puerto Rico site had a greater percentage of viable firm swollen seed than the control in the 5ºC (94.5 vs. 82.9%) and 10ºC (11.3 vs. 2.3%) temperature regimes, while MON 88017 from the Hawaii site had a lower percentage of viable firm swollen seed than the control in the 10ºC (5.2 vs. 14.5%), 5/20ºC (1.7 vs. 6.5%) and the 10/20ºC (0.5 vs. 3.3%) temperature regimes. The values of percent viable firm swollen seed were within the range of the references in all temperature regimes and from each production location, except for the seed produced at the Hawaii site at 10ºC, 20ºC, and 5/20ºC.

E.2.e. Percent Dead Seed

For the seed produced in Puerto Rico and Hawaii, no differences were detected in percent dead seed between MON 88017 and the control in all temperature regimes, except for the Puerto Rico seed at 5ºC (Tables E-1 and E-2). In the 5ºC regime, MON 88017 from Puerto Rico had a lower percent dead seed compared to the control (5.5 vs. 17.1%). The percent dead seed values for MON 88017 were always within the range of values of the reference materials in all temperature regimes from both production locations.

Table E-1. Results of the Germination Testing for Seed Produced at Puerto Rico

Temp. Regime (°C)	Seed Material	Mean[1]				
		Normal Germinated (%)	Viable Hard (%)	Abnormal Germinated (%)	Viable Firm Swollen (%)	Dead (%)
5	MON 88017	0.0	0.0	0.0	94.5*	5.5*
	Control	0.0	0.0	0.0	82.9	17.1
	Reference Range[2]	0-0	0-0	0-0	43-97	3-57
10	MON 88017	0.0	0.0	88.0*	11.3*	0.3
	Control	0.0	0.0	97.8	2.3	0.0
	Reference Range[2]	0-0	0-0	69-100	0-30	0-7
20	MON 88017	98.5	0.0	1.5	0.0	0.0
	Control	98.3	0.0	1.5	0.0	0.3
	Reference Range[2]	90-100	0-0	0-4	0-1	0-6
30	MON 88017	100.0	0.0	0.0	0.0	0.0
	Control	99.3	0.0	0.5	0.0	0.3
	Reference Range[2]	97-100	0-0	0-2	0-0	0-3
5/20	MON 88017	71.8	0.0	27.0	0.8	0.5
	Control	71.5	0.0	28.5	0.0	0.0
	Reference Range[2]	7-100	0-0	0-86	0-6	0-3
10/20	MON 88017	92.3	0.0	7.5	0.3	0.0
	Control	93.0	0.0	6.8	0.0	0.3
	Reference Range[2]	68-100	0-0	0-27	0-5	0-4
20/30	MON 88017	100.0	0.0	0.0	0.0	0.0
	Control	100.0	0.0	0.0	0.0	0.0
	Reference Range[2]	96-100	0-0	0-1	0-1	0-4

* Indicates a significant difference between MON 88017 corn and the control at $p \leq 0.05$.
[1] Mean percent normal germinated, abnormal germinated, dead, viable firm swollen, or viable hard seed.
[2] Minimum and maximum values of combined data for all three reference hybrids.

Table E-2. Results of the Germination Testing for Seed Produced at Hawaii

Temp. Regime (°C)	Seed Material	Mean[1]				
		Normal Germinated (%)	Viable Hard (%)	Abnormal Germinated (%)	Viable Firm Swollen (%)	Dead (%)
5	MON 88017	0.0	0.0	0.0	80.9	19.1
	Control	0.0	0.0	0.0	78.5	21.2
	Reference Range[2]	0-0	0-0	0-0	67-90	10-33
10	MON 88017	0.0	0.0	91.5*	5.2*	1.8
	Control	0.0	0.0	82.5	14.5	3.0
	Reference Range[2]	0-0	0-0	93-100	0-2	0-6
20	MON 88017	96.5	0.0	3.0	0.3	0.3
	Control	96.3	0.0	3.8	0.0	0.0
	Reference Range[2]	92-100	0-0	0-7	0-0	0-5
30	MON 88017	98.8	0.0	1.3	0.0	0.0
	Control	99.3	0.0	0.5	0.0	0.3
	Reference Range[2]	94-100	0-0	0-5	0-0	0-5
5/20	MON 88017	74.0	0.0	24.0	1.7*	0.3
	Control	70.0	0.0	22.5	6.5	1.0
	Reference Range[2]	54-90	0-0	7-45	0-0	0-4
10/20	MON 88017	92.9*	0.0	5.3	0.5*	1.3
	Control	84.3	0.0	11.0	3.3	1.5
	Reference Range[2]	77-96	0-0	2-23	0-1	0-3
20/30	MON 88017	99.3	0.0	0.0	0.0	0.7
	Control	98.3	0.0	1.0	0.0	0.7
	Reference Range[2]	95-100	0-0	0-3	0-0	0-4

* Indicates a significant difference between MON 88017 corn and the control at $p \leq 0.05$.
[1] Mean percent normal germinated, abnormal germinated, dead, viable firm swollen, or viable hard seed.
[2] Minimum and maximum values of combined data for all three reference hybrids.

Appendix F: Phenotypic Assessment: Individual Site Results From 2001 and 2002 Field Trials

The methods and sites for the phenotypic assessments are described in the text in Section VII.

F.1. 2001 Individual Site Results

Discussion of the results is limited to statistically significant differences. If it is stated that the test is different from the control for one or more of the measured characteristics, it implies that the differences were statistically significant at $p \leq 0.05$.

A total of 100 comparisons were made between MON 88017 and the control (11, 12, 13, 14, 12, 13, 14, and 11 characteristics at FL, JE, LA, MN, RA, SC, TL, and YK, respectively, because of the different numbers of characteristics evaluated at each site (Tables F-1 through F-8). There were no differences detected between MON 88017 corn and the control at any of the sites for 10 of the possible 14 phenotypic characteristics (seedling vigor, days to 50% pollen shed, days to 50% silk emergence, plant height, final stand count, dropped ears, stalk lodged plants, root lodged plants, test weight, and yield).

Single differences between MON 88017 and the control were detected at:

- FL for staygreen (Table F-1) with no difference detected at five other sites.
- JE for early stand count (Table F-2) with no difference detected at six other sites.
- TL for ear height (Table F-7) with no difference detected at seven other sites.
- TL for grain moisture (Table F-7) with no difference detected at seven other sites.

The frequency of observed single differences (4/100 = 4%) between the test and the control is less than would be expected due to random experimental effects (5%). The across-sites results and the conclusions drawn from this data are provided in Section VII.A.

Table F-1. Phenotypic Comparison of MON 88017 to the Control at the FL Site

Characteristic	MON 88017	Control
Seedling Vigor	4.8	5.0
Early Stand Count	62.8	65.5
Days to 50% Pollen Shed	70.5	71.0
Days to 50% Silk Emergence	70.0	70.5
Ear Height (in)	47.8	47.0
Plant Height (in)	90.0	89.5
Staygreen	6.0*[1]	7.0*[1]
Final Stand Count	62.0	67.5
Dropped Ears (#/plot)	-	-
Stalk Lodged Plants (#/plot)	-	-
Root Lodged Plants (#/plot)	-	-
Test Weight (lbs/bu)	52.5	51.3
Grain Moisture (%)	28.1	27.7
Yield (bu/a)	110.5	101.9

[1] Mean instead of LSMean reported due to no variation between replications.
* Indicates that MON 88017 corn is statistically different from the control hybrid at $p \leq 0.05$.
- Not collected or not applicable.

Table F-2. Phenotypic Comparison of MON 88017 to the Control at the JE Site

Characteristic	MON 88017	Control
Seedling Vigor	6.3	6.8
Early Stand Count	72.3*	64.3*
Days to 50% Pollen Shed	66.0	66.0
Days to 50% Silk Emergence	66.3	66.0
Ear Height (in)	43.3	43.0
Plant Height (in)	84.8	83.3
Staygreen	-	-
Final Stand Count	56.5	57.8
Dropped Ears (#/plot)	-	-
Stalk Lodged Plants (#/plot)	15.5	8.0
Root Lodged Plants (#/plot)	2.0	2.3
Test Weight (lbs/bu)	51.9	52.8
Grain Moisture (%)	14.7	15.0
Yield (bu/a)	132.8	147.4

* Indicates that MON 88017 corn is statistically different from the control hybrid at $p \leq 0.05$.
- Not collected or not applicable.

Table F-3. Phenotypic Comparison of MON 88017 to the Control at the LA Site

Characteristic	MON 88017	Control
Seedling Vigor	2.8	3.3
Early Stand Count	67.0	64.3
Days to 50% Pollen Shed	62.5	62.0
Days to 50% Silk Emergence	62.0	62.0
Ear Height (in)	44.3	49.0
Plant Height (in)	88.5	89.3
Staygreen	4.5	4.8
Final Stand Count	-	-
Dropped Ears (#/plot)	0.0[1]	0.0[1]
Stalk Lodged Plants (#/plot)	0.0[1]	0.0[1]
Root Lodged Plants (#/plot)	0.0	0.8
Test Weight (lbs/bu)	53.0	52.3
Grain Moisture (%)	23.4	23.2
Yield (bu/a)	166.2	161.5

[1] Mean instead of LSMean reported due to no variation between replications.
- Not collected or not applicable.

Table F-4. Phenotypic Comparison of MON 88017 to the Control at the MN Site

Characteristic	MON 88017	Control
Seedling Vigor	2.5	2.8
Early Stand Count	69.0	59.8
Days to 50% Pollen Shed	68.0	67.0
Days to 50% Silk Emergence	68.0[1]	68.0[1]
Ear Height (in)	39.0	42.5
Plant Height (in)	89.0	88.8
Staygreen	4.3	4.0
Final Stand Count	58.0	58.0
Dropped Ears (#/plot)	0.0[1]	0.0[1]
Stalk Lodged Plants (#/plot)	0.8	0.5
Root Lodged Plants (#/plot)	1.3	0.0
Test Weight (lbs/bu)	61.6	59.4
Grain Moisture (%)	26.3	26.2
Yield (bu/a)	135.4	134.7

[1] Mean instead of LSMean reported because of no variation between replications.
- Not collected or not applicable.

Table F-5. Phenotypic Comparison of MON 88017 to the Control at the RA Site

Characteristic	MON 88017	Control
Seedling Vigor	3.5	4.0
Early Stand Count	58.8	60.3
Days to 50% Pollen Shed	61.5	61.8
Days to 50% Silk Emergence	61.5	61.8
Ear Height (in)	46.3	43.0
Plant Height (in)	88.3	86.5
Staygreen	5.5	6.3
Final Stand Count	-	-
Dropped Ears (#/plot)	2.3	3.8
Stalk Lodged Plants (#/plot)	9.5	11.3
Root Lodged Plants (#/plot)	0.0[1]	0.0[1]
Test Weight (lbs/bu)	-	-
Grain Moisture (%)	19.6	20.2
Yield (bu/a)	136.8	128.6

[1] Mean instead of LSMean reported due to no variation between replications.
- Not collected or not applicable.

Table F-6. Phenotypic Comparison of MON 88017 to the Control at the SC Site

Characteristic	MON 88017	Control
Seedling Vigor	2.8	3.3
Early Stand Count	76.8	79.3
Days to 50% Pollen Shed	68.8	68.5
Days to 50% Silk Emergence	68.8	68.5
Ear Height (in)	44.3	44.5
Plant Height (in)	89.8	89.3
Staygreen	7.8	7.3
Final Stand Count	-	-
Dropped Ears (#/plot)	0.3	0.0
Stalk Lodged Plants (#/plot)	7.5	4.5
Root Lodged Plants (#/plot)	1.5	1.3
Test Weight (lbs/bu)	55.2	55.4
Grain Moisture (%)	20.8	21.2
Yield (bu/a)	160.8	146.6

- Not collected or not applicable.

Table F-7. Phenotypic Comparison of MON 88017 to the Control at the TL Site

Characteristic	MON 88017	Control
Seedling Vigor	3.0[1]	3.0[1]
Early Stand Count	77.0	74.0
Days to 50% Pollen Shed	69.0	68.3
Days to 50% Silk Emergence	69.0	68.0
Ear Height (in)	45.5*	42.8*
Plant Height (in)	90.3	87.5
Staygreen	4.8	4.8
Final Stand Count	70.0[1]	70.0[1]
Dropped Ears (#/plot)	0.8	0.3
Stalk Lodged Plants (#/plot)	4.3	4.8
Root Lodged Plants (#/plot)	0.0[1]	0.0[1]
Test Weight (lbs/bu)	53.3	53.5
Grain Moisture (%)	25.4*	24.0*
Yield (bu/a)	200.4	182.0

[1] Mean instead of LSMean reported due to no variation between replications.
* Indicates that MON 88017 corn is statistically different from the control hybrid at $p \leq 0.05$.
- Not collected or not applicable.

Table F-8. Phenotypic Comparison of MON 88017 to the Control at the YK Site

Characteristic	MON 88017	Control
Seedling Vigor	4.3	4.5
Early Stand Count	-	-
Days to 50% Pollen Shed	68.0	68.3
Days to 50% Silk Emergence	70.0	70.3
Ear Height (in)	53.8	50.5
Plant Height (in)	90.0	89.5
Staygreen	-	-
Final Stand Count	-	-
Dropped Ears (#/plot)	0.0[1]	0.0[1]
Stalk Lodged Plants (#/plot)	0.0[1]	0.0[1]
Root Lodged Plants (#/plot)	0.0[1]	0.0[1]
Test Weight (lbs/bu)	54.3	53.5
Grain Moisture (%)	18.5	18.5
Yield (bu/a)	183.8	167.1

[1] Mean instead of LSMean reported due to no variation between replications.
- Not collected or not applicable.

F.2. 2002 Field Trial Results

In the within-site analysis of MON 88017 compared to the control, 11 significant differences were detected out of 140 comparisons (14 characteristics at ten field sites) conducted at $p \leq 0.05$ (Tables F-9 through F-18). A p-value of 0.05 indicates that any differences between the test and control will be incorrectly declared significant in 5% of the total number of comparisons. In this assessment, a frequency of single differences of 7.9% (11/140 x 100) was slightly above the rate expected due to random experimental effects (5%). However, most of the observed differences occurred for one or two different characteristics per site among seven of the ten sites, with the exception of seedling vigor, which suggests that the transformation process produced no significant impacts on the biological characteristics measured.

There were no differences detected between MON 88017 and the control for any of the measured characteristics at three of the ten sites (CL, RL, and VH) (Tables F-10, F-14, and F-15, respectively). As noted above, a total of 11 differences were detected between MON 88017 and the control among seven of the 14 measured characteristics at the remaining sites. The differences were as follows:

- Seedling vigor for MON 88017 was greater compared to the control at MN, WC, and YK (8.0 vs. 5.0, Table F-12; 8.3 vs. 7.3, Table F-16; and 9.0 vs. 8.0, Table F-18, respectively)
- Early stand count for MON 88017 was higher compared to the control at BE and NB (67.0 vs. 61.0 plants/plot, Table F-9 and 67.3 vs. 59.3 plants/plot, Table F-13, respectively) but was lower at WY (67.7 vs. 69.7 plants/plot, Table F-17).
- 50% pollen shed occurred one day earlier for MON 88017 compared to the control (55.0 vs. 56.0 days) at the CR site, (Table F-11).
- Ear height was lower at the WC site (47.1 vs. 50.6 in) (Table F-16) for MON 88017 compared to the control.
- Plant height was lower at the YK site (71.3 vs. 74.2 in) (Table F-18) for MON 88017 compared to the control.
- Fewer stalk lodged plants were detected for MON 88017 compared to the control at WY (0.0 vs. 1.0 plants/plot) (Table F-17).
- Yield was higher for MON 88017 compared to the control at NB (169.0 vs. 137.5 bu/a) (Table F-13).

Trends toward reduced ear and plant height and fewer stalk lodged plants would not contribute to increased plant weed potential. Increases in early stand count or yield would be agronomically desirable, but could indicate increased weed potential if the trait were transferred to a wild relative. However, no consistent trends for changes in these specific characteristics occurred when the data were pooled across all ten sites (see Table VII-5). Thus, the detected differences for these specific characteristics in the within-site analysis are likely due to random experimental effects and are unlikely to be biologically meaningful with respect to plant weed potential of the crop itself, or if the trait were transferred to a wild relative. Further discussion of the across-sites results and the conclusions drawn from these data are provided in Section VII.A.

Table F-9. Phenotypic Comparison of MON 88017 to the Control at the BE Site

Characteristic	MON 88017	Control
Seedling Vigor	7.7	6.3
Early Stand Count	67.0*	61.0
Days to 50% Pollen Shed	64.0	64.0
Days to 50% Silking	62.3	62.7
Ear Height (in)	45.5	46.7
Plant Height (in)	87.5	87.5
Staygreen	5.0	5.0
Final Stand Count	56.3	56.0
Dropped Ears (#/plot)	1.0	0.3
Stalk Lodged Plants (#/plot)	7.3	5.7
Root Lodged Plants (#/plot)	12.3	9.0
Test Weight (lbs/bu)	57.3	56.9
Grain Moisture (%)	15.6	15.4
Yield (bu/a)	111.0	108.8

* Indicates a statistically significant difference between the test and control at $P \leq 0.05$.

Table F-10. Phenotypic Comparison of MON 88017 to the Control at the CL Site

Characteristic	MON 88017	Control
Seedling Vigor	5.3	5.0
Early Stand Count	54.3	54.0
Days to 50% Pollen Shed	57.7	58.0
Days to 50% Silking	58.0	58.3
Ear Height (in)	39.5	39.2
Plant Height (in)	77.7	80.2
Staygreen	5.3	5.7
Final Stand Count	41.7	43.0
Dropped Ears (#/plot)	0.0	0.0
Stalk Lodged Plants (#/plot)	3.3	4.7
Root Lodged Plants (#/plot)	0.0	0.0
Test Weight (lbs/bu)	57.3	56.3
Grain Moisture (%)	16.5	16.1
Yield (bu/a)	80.0	81.7

Table F-11. Phenotypic Comparison of MON 88017 to the Control at the CR Site

Characteristic	MON 88017	Control
Seedling Vigor	6.7	4.0
Early Stand Count	65.7	67.3
Days to 50% Pollen Shed	55.0*	56.0
Days to 50% Silking	59.0	60.3
Ear Height (in)	37.8	39.5
Plant Height (in)	72.4	73.4
Staygreen	7.0	6.7
Final Stand Count	53.0	52.0
Dropped Ears (#/plot)	0.3	0.3
Stalk Lodged Plants (#/plot)	0.0	0.3
Root Lodged Plants (#/plot)	0.0	0.0
Test Weight (lbs/bu)	54.7	54.9
Grain Moisture (%)	19.8	20.5
Yield (bu/a)	83.6	67.5

* Indicates a statistically significant difference between the test and control at P≤0.05.

Table F-12. Phenotypic Comparison of MON 88017 to the Control at the MN Site

Characteristic	MON 88017	Control
Seedling Vigor	8.0*	5.0
Early Stand Count	57.7	54.0
Days to 50% Pollen Shed	60.3	60.3
Days to 50% Silking	59.7	59.7
Ear Height (in)	42.3	44.5
Plant Height (in)	93.7	93.9
Staygreen	5.3	5.7
Final Stand Count	52.7	49.7
Dropped Ears (#/plot)	0.0	0.0
Stalk Lodged Plants (#/plot)	0.0	0.0
Root Lodged Plants (#/plot)	0.0	0.0
Test Weight (lbs/bu)	53.4	54.3
Grain Moisture (%)	20.8	22.3
Yield (bu/a)	205.3	194.3

* Indicates a statistically significant difference between the test and control at P≤0.05.

Table F-13. Phenotypic Comparison of MON 88017 to the Control at the NB Site

Characteristic	MON 88017	Control
Seedling Vigor	9.0	7.7
Early Stand Count	67.3*	59.3
Days to 50% Pollen Shed	42.0	42.3
Days to 50% Silking	43.7	43.7
Ear Height (in)	43.9	44.0
Plant Height (in)	97.5	95.8
Staygreen	2.0	2.3
Final Stand Count	56.0	54.3
Dropped Ears (#/plot)	0.3	0.7
Stalk Lodged Plants (#/plot)	2.7	5.3
Root Lodged Plants (#/plot)	0.0	0.0
Test Weight (lbs/bu)	58.2	57.8
Grain Moisture (%)	16.6	16.5
Yield (bu/a)	169.0*	137.5

* Indicates a statistically significant difference between the test and control at $P \leq 0.05$.

Table F-14. Phenotypic Comparison of MON 88017 to the Control at the RL Site

Characteristic	MON 88017	Control
Seedling Vigor	6.7	6.0
Early Stand Count	66.0	64.7
Days to 50% Pollen Shed	56.0	56.3
Days to 50% Silking	39.3	39.5
Ear Height (in)	39.3	39.5
Plant Height (in)	80.3	78.5
Staygreen	4.7	4.3
Final Stand Count	56.0	56.0
Dropped Ears (#/plot)	0.0	0.7
Stalk Lodged Plants (#/plot)	2.0	2.3
Root Lodged Plants (#/plot)	0.0	0.0
Test Weight (lbs/bu)	56.0	56.0
Grain Moisture (%)	20.7	21.3
Yield (bu/a)	159.8	195.9

Table F-15. Phenotypic Comparison of MON 88017 to the Control at the VH Site

Characteristic	MON 88017	Control
Seedling Vigor	7.7	7.7
Early Stand Count	62.7	63.7
Days to 50% Pollen Shed	59.0	59.0
Days to 50% Silking	60.0	60.3
Ear Height (in)	40.5	41.3
Plant Height (in)	90.3	91.6
Staygreen	3.7	3.7
Final Stand Count	56.0	56.7
Dropped Ears (#/plot)	0.0	0.3
Stalk Lodged Plants (#/plot)	7.7	11.7
Root Lodged Plants (#/plot)	24.7	31.0
Test Weight (lbs/bu)	56.0	55.5
Grain Moisture (%)	15.8	15.7
Yield (bu/a)	228.8	210.2

Table F-16. Phenotypic Comparison of MON 88017 to the Control at the WC Site

Characteristic	MON 88017	Control
Seedling Vigor	8.3*	7.3
Early Stand Count	67.0	65.0
Days to 50% Pollen Shed	64.3	65.0
Days to 50% Silking	65.0	65.0
Ear Height (in)	47.1*	50.6
Plant Height (in)	93.5	96.5
Staygreen	0.0	0.0
Final Stand Count	56.0	56.0
Dropped Ears (#/plot)	2.3	0.8
Stalk Lodged Plants (#/plot)	2.3	3.3
Root Lodged Plants (#/plot)	2.3	0.3
Test Weight (lbs/bu)	58.1	57.8
Grain Moisture (%)	14.6	14.8
Yield (bu/a)	208.8	226.5

* Indicates a statistically significant difference between the test and control at $P \leq 0.05$.

Table F-17. Phenotypic Comparison of MON 88017 to the Control at the WY Site

Characteristic	MON 88017	Control
Seedling Vigor	8.0	8.0
Early Stand Count	67.7*	69.7
Days to 50% Pollen Shed	63.0	63.0
Days to 50% Silking	61.7	61.7
Ear Height (in)	33.1	32.7
Plant Height (in)	58.1	56.4
Staygreen	2.7	2.0
Final Stand Count	54.0	54.0
Dropped Ears (#/plot)	0.7	0.3
Stalk Lodged Plants (#/plot)	0.0*	1.0
Root Lodged Plants (#/plot)	0.0	0.0
Test Weight (lbs/bu)	48.0	47.3
Grain Moisture (%)	27.5	27.8
Yield (bu/a)	100.9	87.8

* Indicates a statistically significant difference between the test and control at P≤0.05.

Table F-18. Phenotypic Comparison of MON 88017 to the Control at the YK Site

Characteristic	MON 88017	Control
Seedling Vigor	9.0*	8.0
Early Stand Count	65.7	60.3
Days to 50% Pollen Shed	66.7	68.0
Days to 50% Silking	69.3	69.3
Ear Height (in)	35.9	35.9
Plant Height (in)	71.3*	74.2
Staygreen	2.7	4.0
Final Stand Count	56.0	52.7
Dropped Ears (#/plot)	0.0	0.0
Stalk Lodged Plants (#/plot)	0.0	0.0
Root Lodged Plants (#/plot)	0.0	0.0
Test Weight (lbs/bu)	49.7	49.2
Grain Moisture (%)	22.1	23.4
Yield (bu/a)	155.0	138.2

* Indicates a statistically significant difference between the test and control at P≤0.05.

F.3. Confirmatory Ecological Interaction Observations From the 2002 T/C/R Production Trials

The table below summarizes the observations from these trials at three sites (see Section VII).

Table F-19. Corn Stressor Monitoring

Month (1st-5th)	Date (mm/dd/yy)	Stressor or Symptom	Level of Stressor [1]	Plot Differences[2]
IA Site				
1st	06/12/02	Cold, Damp	Slight	No
2nd	07/08/02	None	-	-
3rd	08/05/02	None	-	-
4th	09/04/02	None	-	-
5th	10/01/02	None	-	-
IL Site				
1st	06/05/02	None	-	-
2nd	07/19/02	Heat Stress Drought Stress	Moderate Slight	No No
3rd	08/16/02	Heat Stress Drought Stress Common Rust Rootworm Beetles	Slight Moderate Slight Slight	No No No No
4th	09/18/02	Drought Stress Common Rust	Slight Slight	No No
NE Site				
1st	06/12/02	None	-	-
2nd	07/10/02	Grass hoppers Western corn rootworm	Slight Slight	No Yes
3rd	08/08/02	Spider Mites Drought Conditions Adult Corn Rootworm	Moderate Moderate Slight	No No No
4th	09/05/02	Heat Stress Drought Stress	Slight Slight	No No

[1] Level of Stressor: Slight, Moderate or Severe
[2] Differences between test and control plots or test and reference plots.

Table F-19. Corn Stressor Monitoring (Continued)

Month (1st-6th)	Date (mm/dd/yy)	Stressor or Symptom	Level of Stressor [1]	Plot Differences[2]
OH Site:				
1st	06/10/02	None	-	-
2nd	07/01/02	Compaction	Slight	Yes
3rd	07/31/02	Heat Compaction	Slight Moderate	No No
4th	08/22/02	Heat Drought Compaction	Slight Slight Moderate	No No Yes
5th	09/16/02	Heat Drought	Moderate Moderate	No No
6th	10/08/02	Anthracnose	Slight	No

[1] Level of Stressor: Slight, Moderate or Severe
[2] Differences between test and control plots or test and reference plots.

Appendix G. USDA Notifications

Field trials of MON 88017 were conducted in the U.S. since 1999. The protocols for these trials include field performance, agronomics, and generation of field materials and data necessary for this petition. In addition to the phenotypic assessment data provided for MON 88017, observational data on pest and disease stressors were collected from these product development trials. The majority of these final reports have been submitted to the USDA. However, some final reports, mainly from the 2003-2004 season, are still in preparation. A list of trials conducted under USDA notification and the status of the final reports for these trials are provided in Table G-1.

Table G-1. USDA Notifications Approved for MON 88017 and Status of Trials Conducted Under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
2000-2001 Field Trials			
99-342-02n	01/07/2000	HI, IL, PR	Report Submitted to USDA
00-082-10n	04/21/2000	IA, IL, KS, NE, OH	Report Submitted to USDA
00-090-01n	05/05/2000	HI, PR	Report Submitted to USDA
00-090-02n	05/15/2000	IA, IL, IN	Report Submitted to USDA
00-090-03n	05/15/2000	MN, MO, NE, SD	Report Submitted to USDA
2001-2002 Field Trials			
00-339-02n	01/03/2001	TX	Report Submitted to USDA
00-348-02n	01/30/2001	CA, FL, HI, PR	Report Submitted to USDA
00-356-11n	01/29/2001	TN	Report Submitted to USDA
01-019-11n	02/18/2001	IA, IL	Report Submitted to USDA
01-022-05n	02/21/2001	MO	Report Submitted to USDA
01-022-08n	03/12/2001	MN	Report Submitted to USDA
01-022-09n	02/21/2001	NE	Report Submitted to USDA
01-022-12n	02/21/2001	TX	Report Submitted to USDA
01-022-14n	02/21/2001	SD	Report Submitted to USDA
01-023-08n	02/22/2001	IA	Report Submitted to USDA
01-023-09n	02/23/2001	IN, MO, OH, WI	Report Submitted to USDA
01-023-10n	03/12/2001	KS, MN, NE, SD	Report Submitted to USDA
01-023-11n	02/22/2001	CO	Report Submitted to USDA
01-023-12n	03/08/2001	NE	Report Submitted to USDA
01-023-15n	02/22/2001	HI	Report Submitted to USDA
01-023-16n	02/22/2001	IN	Report Submitted to USDA
01-023-17n	02/22/2001	IA	Report Submitted to USDA
01-024-07n	02/23/2001	IL	Report Submitted to USDA
01-026-17n	02/25/2001	IL	Report Submitted to USDA
01-026-18n	02/25/2001	IA	Report Submitted to USDA
01-026-19n	02/25/2001	IN	Report Submitted to USDA
01-026-20n	02/28/2001	MN	Report Submitted to USDA
01-043-04n	03/14/2001	IA, IL, NE, OH	Report Submitted to USDA
01-051-26n	03/22/2001	IL	Report Submitted to USDA
01-127-02n	06/20/2001	HI	Report Submitted to USDA
01-197-05n	08/22/2001	CA	Report Submitted to USDA
01-197-06n	08/22/2001	HI	Report Submitted to USDA
01-197-07n	08/22/2001	PR	Report Submitted to USDA
01-242-02n	10/17/2001	HI	Report Submitted to USDA
01-256-04n	10/15/2001	HI	Report Submitted to USDA

Table G-1 (cont'd). USDA Notifications Approved for MON 88017 and Status of Trials Conducted Under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
2002-2003 Field Trials			
01-354-04n	01/19/2002	AL, MS	Report Submitted to USDA
02-007-02n	02/06/2002	MO	Report Submitted to USDA
02-007-03n	02/06/2002	IL	Report Submitted to USDA
02-007-04n	02/06/2002	IA	Report Submitted to USDA
02-007-05n	02/06/2002	IN	Report Submitted to USDA
02-007-07n	02/06/2002	MD	Report Submitted to USDA
02-008-07n	02/07/2002	OH	Report Submitted to USDA
02-008-13n	02/07/2002	WI	Report Submitted to USDA
02-008-14n	02/07/2002	PA	Report Submitted to USDA
02-008-24n	02/07/2002	IL	Report Submitted to USDA
02-008-25n	02/07/2002	IA	Report Submitted to USDA
02-008-26n	02/07/2002	SD	Report Submitted to USDA
02-008-27n	02/12/2002	MN	Report Submitted to USDA
02-008-28n	02/07/2002	MO	Report Submitted to USDA
02-009-01n	02/08/2002	OH	Report Submitted to USDA
02-009-02n	02/08/2002	IN	Report Submitted to USDA
02-009-03n	02/08/2002	WI	Report Submitted to USDA
02-009-04n	02/08/2002	NE	Report Submitted to USDA
02-009-06n	02/08/2002	KS	Report Submitted to USDA
02-009-07n	02/08/2002	TN	Report Submitted to USDA
02-009-08n	02/08/2002	NE	Report Submitted to USDA
02-010-12n	02/09/2002	MO	Report Submitted to USDA
02-010-13n	02/09/2002	IL	Report Submitted to USDA
02-010-14n	02/09/2002	NE	Report Submitted to USDA
02-010-15n	02/09/2002	SD	Report Submitted to USDA
02-010-17n	02/12/2002	MN	Report Submitted to USDA
02-014-04n	02/13/2002	SD	Report Submitted to USDA
02-014-05n	02/13/2002	MN	Report Submitted to USDA
02-014-06n	02/13/2002	MO	Report Submitted to USDA
02-014-07n	02/13/2002	IL	Report Submitted to USDA
02-014-08n	02/13/2002	IN	Report Submitted to USDA
02-014-09n	02/13/2002	OH	Report Submitted to USDA
02-014-10n	02/13/2002	WI	Report Submitted to USDA
02-014-11n	03/12/2002	PR	Report Submitted to USDA
02-014-12n	03/12/2002	CA	Report Submitted to USDA
02-015-01n	02/26/2002	KS	Report Submitted to USDA
02-015-02n	02/14/2002	IA	Report Submitted to USDA
02-015-03n	03/12/2002	HI	Report Submitted to USDA
02-022-17n	03/01/2002	MN	Report Submitted to USDA
02-022-27n	02/21/2002	IA	Report Submitted to USDA
02-022-29n	02/21/2002	KS	Report Submitted to USDA
02-022-30n	02/21/2002	IL	Report Submitted to USDA

Table G-1 (cont'd). USDA Notifications Approved for MON 88017 and Status of Trials Conducted Under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
02-022-31n	02/21/2002	IA	Report Submitted to USDA
02-022-32n	02/21/2002	SD	Report Submitted to USDA
02-022-33n	02/21/2002	MO	Report Submitted to USDA
02-022-34n	02/21/2002	IN	Report Submitted to USDA
02-022-35n	02/21/2002	OH	Report Submitted to USDA
02-022-36n	02/21/2002	WI	Report Submitted to USDA
02-022-47n	02/21/2002	KS	Report Submitted to USDA
02-022-48n	02/21/2002	IL	Report Submitted to USDA
02-022-49n	02/21/2002	IN	Report Submitted to USDA
02-022-51n	02/21/2002	NE	Report Submitted to USDA
02-022-52n	02/21/2002	IL	Report Submitted to USDA
02-023-02n	02/22/2002	IA	Report Submitted to USDA
02-023-03n	02/22/2002	IL	Report Submitted to USDA
02-023-04n	02/22/2002	NE	Report Submitted to USDA
02-023-05n	02/22/2002	KS	Report Submitted to USDA
02-023-06n	02/22/2002	NE	Report Submitted to USDA
02-023-07n	03/20/2002	TN	Report Submitted to USDA
02-023-09n	02/22/2002	NE	Report Submitted to USDA
02-023-10n	02/22/2002	OH	Report Submitted to USDA
02-028-11n	02/27/2002	SD	Report Submitted to USDA
02-028-12n	03/01/2002	MN	Report Submitted to USDA
02-028-13n	02/27/2002	IA	Report Submitted to USDA
02-028-14n	02/27/2002	MO	Report Submitted to USDA
02-028-15n	02/27/2002	IL	Report Submitted to USDA
02-028-16n	02/27/2002	IN	Report Submitted to USDA
02-028-17n	02/27/2002	OH	Report Submitted to USDA
02-028-18n	02/27/2002	WI	Report Submitted to USDA
02-028-19n	03/12/2002	HI	Report Submitted to USDA
02-028-20n	03/12/2002	PR	Report Submitted to USDA
02-028-21n	03/12/2002	CA	Report Submitted to USDA
02-030-02n	03/01/2002	IA	Report Submitted to USDA
02-030-03n	03/01/2002	IL	Report Submitted to USDA
02-031-03n	03/12/2002	HI	Report Submitted to USDA
02-031-04n	03/02/2002	NE	Report Submitted to USDA
02-031-05n	03/02/2002	MN	Report Submitted to USDA
02-031-06n	03/02/2002	IN	Report Submitted to USDA
02-031-07n	03/02/2002	IA	Report Submitted to USDA
02-036-03n	03/07/2002	IA	Report Submitted to USDA
02-036-04n	04/10/2002	MN	Report Submitted to USDA
02-036-05n	03/07/2002	NE	Report Submitted to USDA
02-036-06n	03/07/2002	WI	Report Submitted to USDA

Table G-1 (cont'd). USDA Notifications Approved for MON 88017 and Status of Trials Conducted Under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
02-036-09n	03/07/2002	KS	Report Submitted to USDA
02-036-15n	03/07/2002	MO	Report Submitted to USDA
02-036-17n	03/07/2002	IA	Report Submitted to USDA
02-037-04n	03/08/2002	IN	Report Submitted to USDA
02-037-07n	03/18/2002	IL	Report Submitted to USDA
02-037-08n	03/08/2002	KS	Report Submitted to USDA
02-042-17n	03/13/2002	IL	Report Submitted to USDA
02-042-18n	03/13/2002	MO	Report Submitted to USDA
02-043-04n	03/14/2002	IL	Report Submitted to USDA
02-051-18n	03/22/2002	IL	Report Submitted to USDA
02-066-14n	04/10/2002	IL	Report Submitted to USDA
02-070-27n	04/26/2002	HI	Report Submitted to USDA
02-092-07n	05/02/2002	NE	Report Submitted to USDA
02-092-08n	05/02/2002	IA	Report Submitted to USDA
02-115-04n	05/30/2002	HI	Report Submitted to USDA
02-213-05n	09/10/2002	PR	Report Submitted to USDA
02-213-08n	09/13/2002	HI	Report Submitted to USDA
02-214-04n	09/12/2002	CA	Report Submitted to USDA
02-247-05n	10/15/2002	PR	Report Submitted to USDA
02-309-04n	12/05/2002	HI	Report Submitted to USDA
2003-2004 Field Trials			
03-015-02n	02/14/2003	IA	Report Submitted to USDA
03-015-03n	02/14/2003	HI	Report Submitted to USDA
03-015-04n	02/14/2003	HI	In Progress
03-015-05n	02/14/2003	IN	In Progress
03-015-06n	02/14/2003	NE	Report Submitted to USDA
03-015-11n	02/14/2003	HI	In Progress
03-021-08n	02/20/2003	HI	In Progress
03-021-09n	02/20/2003	HI	In Progress
03-021-10n	02/20/2003	IN	Report Submitted to USDA
03-021-11n	02/20/2003	PR	In Progress
03-021-12n	02/20/2003	PR	Report Submitted to USDA
03-021-13n	02/20/2003	HI	In Progress
03-022-02n	02/21/2003	HI	In Progress
03-022-05n	02/21/2003	HI	In Progress
03-023-12n	04/17/2003	WI	In Progress
03-027-05n	03/28/2003	HI	In Progress
03-030-04n	03/01/2003	IL	In Progress
03-030-08n	03/01/2003	IL	In Progress
03-030-09n	03/01/2003	IA	In Progress

Table G-1 (cont'd). USDA Notifications Approved for MON 88017 and Status of Trials Conducted Under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
03-030-13n	03/01/2003	SD	In Progress
03-030-14n	03/01/2003	IA	In Progress
03-030-15n	03/01/2003	WI	In Progress
03-030-17n	03/01/2003	IN	In Progress
03-030-18n	03/01/2003	MO	In Progress
03-034-12n	03/05/2003	IN	In Progress
03-034-13n	03/05/2003	WI	In Progress
03-034-14n	03/05/2003	IA	In Progress
03-034-15n	03/05/2003	WI	In Progress
03-034-16n	03/05/2003	IN	In Progress
03-034-17n	03/05/2003	KS	In Progress
03-034-18n	03/11/2003	MN	In Progress
03-034-19n	03/05/2003	NE	In Progress
03-034-20n	03/05/2003	OH	In Progress
03-034-21n	03/05/2003	MO	In Progress
03-034-22n	04/03/2003	MI	In Progress
03-034-23n	03/05/2003	IL	In Progress
03-034-29n	03/05/2003	IA	In Progress
03-034-31n	03/05/2003	IL	In Progress
03-034-32n	03/12/2003	MN	In Progress
03-035-01n	03/06/2003	IA	In Progress
03-035-02n	03/06/2003	KS	In Progress
03-035-03n	04/03/2003	MI	In Progress
03-035-04n	03/06/2003	MO	In Progress
03-035-05n	03/06/2003	IA	In Progress
03-035-06n	03/26/2003	IA	In Progress
03-035-07n	03/06/2003	IA	In Progress
03-035-08n	03/06/2003	IL	In Progress
03-037-01n	03/08/2003	IL	In Progress
03-037-02n	03/08/2003	KS	In Progress
03-037-03n	03/12/2003	MN	In Progress
03-037-04n	03/08/2003	MO	In Progress
03-037-05n	03/08/2003	NE	In Progress
03-037-09n	03/12/2003	NE	In Progress
03-042-08n	03/13/2003	PA	In Progress
03-042-16n	04/16/2003	MI	In Progress
03-042-18n	03/13/2003	IL	In Progress
03-043-01n	03/17/2003	OH	In Progress
03-043-03n	03/17/2003	TN	In Progress
03-083-01n	04/30/2003	IL	In Progress
03-098-05n	05/08/2003	IL	In Progress
03-112-04n	05/22/2003	IL	In Progress
03-112-08n	05/22/2003	HI	In Progress

Table G-1 (cont'd). USDA Notifications Approved for MON 88017 and Status of Trials Conducted Under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
03-112-09n	05/22/2003	IA	In Progress
03-112-10n	05/22/2003	PR	In Progress
03-112-14n	05/22/2003	IL	In Progress
03-184-02n	08/02/2003	TX	In Progress
03-219-05n	10/08/2003	HI	In Progress
03-230-01n	09/17/2003	PR	In Progress
03-259-02n	10/16/2003	HI	In Progress
03-279-02n	11/12/2003	PR	In Progress
03-266-02n	11/21/2003	HI	In Progress
03-272-06n	11/21/2003	HI	In Progress
03-301-09n	11/27/2003	PR	In Progress
03-301-10n	11/27/2003	HI	In Progress
03-301-11n	11/27/2003	HI	In Progress
03-301-12n	12/01/2003	HI	In Progress
03-301-13n	11/27/2003	HI	In Progress
03-301-14n	11/27/2003	HI	In Progress
2004 Field Trials			
03-322-02n	01/21/2004	CA	In Progress
03-322-03n	01/21/2004	HI	In Progress
03-356-01n	02/03/2004	HI	In Progress
03-356-02n	01/21/2004	TX	In Progress
03-356-04n	02/03/2004	PR	In Progress
04-006-03n	02/05/2004	PR	In Progress
04-014-06n	02/13/2004	TN	In Progress
04-021-04n	02/20/2004	IA	In Progress
04-021-06n	02/20/2004	IA	In Progress
04-021-07n	02/20/2004	MI	In Progress
04-021-08n	02/20/2004	MO	In Progress
04-022-01n	02/25/2004	IA	In Progress
04-022-02n	02/25/2004	NE	In Progress
04-023-01n	02/25/2004	IN	In Progress
04-023-02n	02/25/2004	KS	In Progress
04-023-03n	02/25/2004	WI	In Progress
04-023-05n	02/25/2004	IN	In Progress
04-023-06n	02/25/2004	MO	In Progress
04-023-07n	02/25/2004	IA	In Progress
04-023-08n	02/25/2004	NE	In Progress
04-028-11n	02/27/2004	IA	In Progress
04-028-12n	02/27/2004	IL	In Progress
04-028-13n	02/27/2004	IN	In Progress
04-028-14n	03/26/2004	MI	In Progress
04-028-15n	02/27/2004	NE	In Progress

Table G-1 (cont'd). USDA Notifications Approved for MON 88017 and Status of Trials Conducted Under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
04-028-16n	02/27/2004	IL	In Progress
04-028-18n	02/27/2004	GA	Canceled
04-028-19n	02/27/2004	MI	In Progress
04-028-21n	02/27/2004	IL	In Progress
04-028-22n	02/27/2004	WI	Canceled
04-028-34n	02/27/2004	KS	In Progress
04-028-35n	04/02/2004	IA	In Progress
04-028-47n	02/27/2004	OH	In Progress
04-028-48n	04/02/2004	HI	In Progress
04-028-49n	02/27/2004	PR	In Progress
04-028-50n	02/27/2004	IA	In Progress
04-028-51n	02/27/2004	IL	In Progress
04-028-52n	03/05/2004	NE	In Progress
04-030-03n	03/05/2004	SD	In Progress
04-035-03n	04/02/2004	IL	In Progress
04-035-04n	04/02/2004	IN	In Progress
04-035-05n	03/05/2004	OH	In Progress
04-036-05n	03/06/2004	WI	In Progress
04-037-04n	03/26/2004	MN	In Progress
04-037-05n	03/12/2004	IL	In Progress
04-037-06n	03/07/2004	IL	In Progress
04-037-07n	03/12/2004	IA	In Progress
04-037-08n	03/07/2004	NE	In Progress
04-037-09n	03/07/2004	KS	In Progress
04-037-10n	03/07/2004	OH	In Progress
04-037-11n	03/07/2004	IN	In Progress
04-041-06n	03/11/2004	IN	In Progress
04-041-07n	03/11/2004	IL	In Progress
04-041-08n	03/11/2004	IL	In Progress
04-042-15n	03/26/2004	MN	In Progress
04-048-05n	04/02/2004	IA	In Progress
04-055-05n	03/25/2004	IL	In Progress

Appendix H: Compositional Analysis Tables

Composition of grain and forage of MON 88107 was analyzed and compared to conventional corn. For this analysis, MON 88017 and conventional corn plants were grown at replicated field trials at three locations: NE, IA and IL. Analysis of grain included proximates (protein, fat, ash and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), amino acids, fatty acids, minerals (calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium and zink), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic, and p-coumaric acid), and carbohydrates by calculation. Analysis of forage included proximates, ADF, NDF, minerals (calcium and phosphorus), and carbohydrates by calculation. Established methods were used for the analysis of compositional components and are described in the safety, compostional and nutritional submission made to the FDA on March 31, 2004.

The results of compositional analysis, including a summary of statistical differences, are discussed in Section VII.B. Tables H-1 to H-7 provide a summary of the combined site analysis, and Table H-8 lists the literature and historical ranges for the components analyzed.

Table H-1. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Ash (% dwt)	3.99 ± 0.24 (3.30 - 5.53)	4.04 ± 0.24 (3.59 - 4.67)	-0.051 ± 0.28 (-1.37 – 1.55)	-0.74,0.64	0.861	(2.62 - 6.78) [0.72,7.42]
Carbohydrates (% dwt)	86.19 ± 0.62 (83.54 - 87.88)	86.48 ± 0.62 (84.43 - 87.71)	-0.29 ± 0.40 (-2.58 – 1.73)	-1.11,0.54	0.478	(81.86 - 89.90) [78.70,93.43]
Fat, total (% dwt)	1.61 ± 0.29 (0.80 - 3.13)	1.65 ± 0.29 (0.83 - 2.97)	-0.039 ± 0.25 (-1.47 – 1.99)	-0.56,0.48	0.878	(0.69 - 2.92) [0.80,2.95]
Moisture (% fw)	70.86 ± 0.66 (68.50 - 72.70)	70.66 ± 0.66 (69.10 - 72.70)	0.20 ± 0.39 (-1.40 – 1.90)	-0.61,1.01	0.615	(65.20 - 78.60) [59.37,80.83]
Protein (% dwt)	8.20 ± 0.31 (7.44 - 8.97)	7.82 ± 0.31 (6.79 - 8.54)	0.38 ± 0.25 (-0.99 – 1.65)	-0.13,0.88	0.137	(6.31 - 9.96) [4.17,11.81]
ADF (% dwt)	26.54 ± 1.25 (24.29 - 29.97)	25.45 ± 1.25 (23.34 - 28.13)	1.10 ± 1.76 (-2.58 – 4.08)	-2.97,5.16	0.549	(19.16 - 35.55) [13.95,38.96]
NDF (% dwt)	37.34 ± 1.22 (33.44 - 45.05)	38.33 ± 1.22 (35.86 - 41.18)	-0.99 ± 1.42 (-4.63 – 6.97)	-3.90,1.91	0.490	(30.27 - 57.93) [23.80,54.73]
Calcium (% dwt)	0.22 ± 0.014 (0.19 - 0.26)	0.23 ± 0.014 (0.18 - 0.31)	-0.0092 ± 0.014 (-0.054 – 0.024)	-0.044,0.026	0.542	(0.13 - 0.32) [0.11,0.32]
Phosphorus (% dwt)	0.25 ± 0.011 (0.21 - 0.30)	0.25 ± 0.011 (0.20 - 0.30)	0.0017 ± 0.013 (-0.060 - 0.079)	-0.029,0.032	0.899	(0.16 - 0.31) [0.095,0.38]

[a]dwt=dry weight; ADF=acid detergent fiber; NDF=neutral detergent fiber; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table H-2. Comparison of the Amino Acid Content in Grain from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Alanine (% total AA)	7.55 ± 0.084 (7.29 - 7.70)	7.55 ± 0.084 (7.34 - 7.79)	-0.0026 ± 0.039 (-0.19 - 0.18)	-0.097,0.092	0.949	(7.24 - 8.16) [6.66,8.49]
Arginine (% total AA)	4.42 ± 0.11 (4.10 - 4.74)	4.29 ± 0.11 (4.01 - 4.63)	0.13 ± 0.060 (-0.12 - 0.36)	-0.013,0.28	0.066	(3.72 - 5.08) [3.34,5.67]
Aspartic acid (% total AA)	6.22 ± 0.050 (6.09 - 6.34)	6.25 ± 0.050 (6.04 - 6.45)	-0.032 ± 0.067 (-0.34 - 0.18)	-0.20,0.13	0.648	(6.18 - 6.81) [5.77,7.16]
Cystine (% total AA)	2.14 ± 0.054 (1.93 - 2.26)	2.15 ± 0.054 (1.93 - 2.30)	-0.013 ± 0.042 (-0.20 - 0.17)	-0.098,0.073	0.766	(1.82 - 2.58) [1.46,2.89]
Glutamic acid (% total AA)	20.40 ± 0.18 (19.80 - 20.87)	20.44 ± 0.18 (19.91 - 20.84)	-0.036 ± 0.086 (-0.52 - 0.48)	-0.25,0.17	0.686	(19.46 - 21.57) [18.01,22.15]
Glycine (% total AA)	3.45 ± 0.063 (3.32 - 3.62)	3.45 ± 0.063 (3.18 - 3.61)	0.0061 ± 0.031 (-0.081 - 0.19)	-0.058,0.070	0.844	(3.29 - 4.03) [2.81,4.54]
Histidine (% total AA)	2.99 ± 0.049 (2.90 - 3.10)	2.95 ± 0.049 (2.83 - 3.14)	0.032 ± 0.022 (-0.056 - 0.10)	-0.023,0.087	0.200	(2.50 - 3.12) [2.16,3.60]
Isoleucine (% total AA)	3.59 ± 0.037 (3.43 - 3.71)	3.57 ± 0.037 (3.45 - 3.76)	0.025 ± 0.044 (-0.15 - 0.25)	-0.065,0.11	0.577	(3.39 - 3.79) [3.30,3.84]
Leucine (% total AA)	13.28 ± 0.20 (12.69 - 13.62)	13.31 ± 0.20 (12.76 - 14.11)	-0.037 ± 0.098 (-0.69 - 0.56)	-0.28,0.20	0.717	(12.11 - 14.35) [10.72,15.18]

[a]AA=amino acids; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval
[c]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table H-2 (cont). Comparison of the Amino Acid Content in grain from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Lysine (% total AA)	2.69 ± 0.058 (2.42 - 2.87)	2.66 ± 0.058 (2.49 - 2.82)	0.024 ± 0.047 (-0.072 - 0.11)	-0.074,0.12	0.614	(2.44 - 3.27) [2.06,3.73]
Methionine (% total AA)	1.98 ± 0.059 (1.85 - 2.05)	2.01 ± 0.059 (1.83 - 2.20)	-0.030 ± 0.043 (-0.15 - 0.12)	-0.14,0.076	0.515	(1.70 - 2.47) [1.37,2.60]
Phenylalanine (% total AA)	5.18 ± 0.059 (4.97 - 5.31)	5.14 ± 0.059 (5.01 - 5.32)	0.035 ± 0.055 (-0.13 - 0.25)	-0.10,0.17	0.545	(4.82 - 5.39) [4.57,5.71]
Proline (% total AA)	9.39 ± 0.094 (9.02 - 9.69)	9.34 ± 0.094 (8.85 - 9.80)	0.046 ± 0.11 (-0.61 - 0.71)	-0.18,0.27	0.676	(8.35 - 9.72) [7.60,10.37]
Serine (% total AA)	4.83 ± 0.049 (4.65 - 5.04)	4.91 ± 0.049 (4.63 - 5.13)	-0.081 ± 0.068 (-0.47 - 0.42)	-0.22,0.059	0.244	(4.81 - 5.23) [4.60,5.43]
Threonine (% total AA)	3.22 ± 0.040 (3.10 - 3.38)	3.25 ± 0.040 (3.06 - 3.37)	-0.026 ± 0.045 (-0.25 - 0.24)	-0.12,0.067	0.572	(2.96 - 3.55) [2.89,3.84]
Tryptophan (% total AA)	0.54 ± 0.027 (0.48 - 0.60)	0.55 ± 0.027 (0.41 - 0.68)	-0.0090 ± 0.018 (-0.17 - 0.096)	-0.046,0.028	0.627	(0.44 - 0.83) [0.36,0.77]
Tyrosine (% total AA)	3.35 ± 0.16 (2.35 - 3.66)	3.43 ± 0.16 (2.58 - 3.66)	-0.079 ± 0.23 (-1.18 - 0.98)	-0.61,0.46	0.743	(2.26 - 3.80) [2.62,4.26]
Valine (% total AA)	4.79 ± 0.039 (4.60 - 4.92)	4.74 ± 0.039 (4.60 - 4.94)	0.043 ± 0.052 (-0.25 - 0.26)	-0.064,0.15	0.414	(4.44 - 5.04) [4.22,5.27]

[a]AA=amino acids; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval
[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to ro.

Table H-3. Comparison of the Fatty Acid Content in Grain from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
16:0 palmitic (% total FA)	10.24 ± 0.43 (10.07 - 10.52)	11.27 ± 0.43 (10.14 - 14.57)	-1.03 ± 0.60 (-4.35 - 0.36)	-2.42,0.37	0.128	(9.29 - 17.81) [6.51,16.50]
16:1 pamitoleic (% total FA)	0.18 ± 0.010 (0.16 - 0.21)	0.18 ± 0.010 (0.16 - 0.22)	-0.0030 ± 0.0064 (-0.029 - 0.025)	-0.019,0.013	0.655	(0.054 - 0.21) [0.0017,0.28]
18:0 stearic (% total FA)	2.01 ± 0.073 (1.80 - 2.19)	2.07 ± 0.073 (1.76 - 2.23)	-0.052 ± 0.046 (-0.28 - 0.25)	-0.15,0.042	0.266	(1.68 - 2.30) [1.41,2.53]
18:1 oleic (% total FA)	22.74 ± 0.23 (22.20 - 23.53)	22.87 ± 0.23 (21.43 - 23.51)	-0.13 ± 0.24 (-0.94 - 1.13)	-0.71,0.46	0.613	(19.79 - 34.46) [9.25,44.14]
18:2 linoleic (% total FA)	62.85 ± 0.39 (61.86 - 63.72)	61.52 ± 0.39 (59.10 - 63.18)	1.34 ± 0.53 (-0.64 - 4.19)	0.093,2.58	0.038	(51.64 - 64.12) [41.22,74.09]
18:3 linolenic (% total FA)	1.21 ± 0.062 (1.15 - 1.26)	1.32 ± 0.062 (1.19 - 1.77)	-0.11 ± 0.077 (-0.53 - 0.043)	-0.30,0.079	0.205	(0.84 - 1.91) [0.42,1.95]
20:0 arachidic (% total FA)	0.37 ± 0.010 (0.35 - 0.39)	0.38 ± 0.010 (0.35 - 0.41)	-0.0085 ± 0.0032 (-0.028 - 0.0088)	-0.015,-0.0019	0.012	(0.36 - 0.45) [0.31,0.49]
20:1 eicosenoic (% total FA)	0.24 ± 0.0056 (0.23 - 0.26)	0.25 ± 0.0056 (0.24 - 0.26)	-0.0034 ± 0.0034 (-0.019 - 0.019)	-0.010,0.0036	0.323	(0.24 - 0.36) [0.18,0.40]
22:0 behenic (% total FA)	0.15 ± 0.0027 (0.14 - 0.16)	0.15 ± 0.0027 (0.14 - 0.17)	-0.0062 ± 0.0038 (-0.018 - 0.014)	-0.014,0.0016	0.116	(0.074 - 0.24) [0.071,0.25]

[a]FA=fatty acids; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval
[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table H-4. Comparison of the Mineral Content in Grain from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Calcium (% dwt)	0.0054 ± 0.00035 (0.0047 - 0.0060)	0.0058 ± 0.00035 (0.0049 - 0.0069)	-0.00040 ± 0.00025 (-0.0013 - -0.00006)	-0.0010,0.00021	0.159	(0.0032 - 0.0060) [0.0017,0.0062]
Copper (mg/kg dwt)	1.73 ± 0.086 (1.48 - 2.05)	1.99 ± 0.086 (1.64 - 2.63)	-0.26 ± 0.12 (-0.95 - 0.41)	-0.54,0.016	0.061	(1.01 - 2.34) [0.17,3.00]
Iron (mg/kg dwt)	21.51 ± 0.59 (20.07 - 22.92)	21.84 ± 0.59 (20.31 - 23.93)	-0.33 ± 0.62 (-2.16 - 2.12)	-1.60,0.93	0.595	(16.42 - 26.03) [12.60,31.26]
Magnesium (% dwt)	0.14 ± 0.0034 (0.13 - 0.15)	0.14 ± 0.0034 (0.13 - 0.16)	-0.0022 ± 0.0044 (-0.024 - 0.018)	-0.011,0.0069	0.618	(0.10 - 0.14) [0.088,0.16]
Manganese (mg/kg dwt)	9.72 ± 0.38 (9.01 - 10.76)	9.37 ± 0.38 (7.55 - 10.44)	0.35 ± 0.38 (-0.39 - 1.56)	-0.57,1.27	0.384	(4.96 - 9.81) [2.45,10.60]
Phosphorus (% dwt)	0.39 ± 0.010 (0.37 - 0.41)	0.39 ± 0.010 (0.36 - 0.43)	-0.0042 ± 0.013 (-0.052 - 0.042)	-0.032,0.023	0.754	(0.28 - 0.41) [0.24,0.44]
Potassium (% dwt)	0.41 ± 0.012 (0.39 - 0.44)	0.42 ± 0.012 (0.38 - 0.47)	-0.0063 ± 0.012 (-0.052 - 0.037)	-0.030,0.018	0.592	(0.29 - 0.43) [0.27,0.48]
Zinc (mg/kg dwt)	24.53 ± 0.98 (22.31 - 27.27)	24.92 ± 0.98 (22.02 - 27.18)	-0.39 ± 0.62 (-3.87 - 1.90)	-1.67,0.89	0.534	(17.15 - 26.18) [13.42,31.37]

[a]dwt=dry weight; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval
[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table H-5. Comparison of the Proximates and Fiber Content in Grain from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Ash (% dwt)	1.54 ± 0.077 (1.31 - 1.68)	1.59 ± 0.077 (1.23 - 1.97)	-0.049 ± 0.087 (-0.45 - 0.43)	-0.23,0.13	0.573	(1.04 - 1.86) [0.94,1.73]
Carbohydrates (% dwt)	82.32 ± 0.40 (81.61 - 83.39)	82.33 ± 0.40 (80.67 - 83.62)	-0.019 ± 0.25 (-1.39 - 0.94)	-0.62,0.58	0.940	(81.46 - 86.68) [79.39,89.67]
Fat, total (% dwt)	3.64 ± 0.13 (3.44 - 3.96)	3.79 ± 0.13 (3.53 - 4.36)	-0.16 ± 0.080 (-0.63 - 0.15)	-0.35,0.041	0.100	(2.38 - 4.43) [0.74,6.01]
Moisture (% fw)	11.10 ± 0.99 (9.03 - 13.20)	11.60 ± 0.99 (9.73 - 14.20)	-0.49 ± 0.35 (-1.10 - -0.10)	-1.36,0.37	0.212	(9.15 - 14.90) [4.67,17.56]
Protein (% dwt)	12.51 ± 0.35 (11.63 - 13.00)	12.28 ± 0.35 (11.22 - 13.82)	0.23 ± 0.24 (-0.82 - 1.37)	-0.36,0.82	0.379	(9.26 - 13.37) [6.20,15.35]
ADF (% dwt)	3.77 ± 0.16 (3.31 - 4.40)	3.54 ± 0.16 (2.97 - 4.69)	0.23 ± 0.18 (-0.62 - 1.16)	-0.13,0.59	0.203	(2.39 - 4.89) [1.89,5.23]
NDF (% dwt)	12.44 ± 0.62 (10.99 - 13.58)	11.87 ± 0.62 (10.38 - 14.29)	0.57 ± 0.50 (-1.21 - 2.64)	-0.66,1.79	0.299	(8.41 - 16.54) [3.51,21.65]
TDF (% dwt)	16.24 ± 0.71 (13.57 - 18.64)	15.40 ± 0.71 (13.18 - 17.84)	0.84 ± 0.96 (-2.39 - 4.19)	-1.51,3.20	0.414	(11.80 - 23.04) [5.72,27.10]

[a]ADF=acid detergent fiber; NDF=neutral detergent fiber; TDF=total dietary fiber; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table H-6. Comparison of the Vitamin Content in Grain from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Folic acid (mg/kg dwt)	0.48 ± 0.021 (0.38 - 0.60)	0.48 ± 0.021 (0.42 - 0.59)	0.0012 ± 0.030 (-0.074 - 0.11)	-0.072,0.075	0.969	(0.28 - 0.61) [0.12,0.77]
Niacin (mg/kg dwt)	20.94 ± 1.20 (17.04 - 24.14)	21.75 ± 1.20 (19.08 - 23.92)	-0.81 ± 0.42 (-2.04 - 0.23)	-1.67,0.050	0.063	(14.11 - 27.77) [3.19,34.49]
Vitamin B_1 (mg/kg dwt)	2.47 ± 0.14 (2.30 - 2.69)	3.24 ± 0.14 (2.99 - 3.60)	-0.77 ± 0.12 (-1.02 - -0.35)	-1.06,-0.48	<0.001	(2.69 - 3.73) [1.96,4.38]
Vitamin B_2 (mg/kg dwt)	1.10 ± 0.041 (0.98 - 1.22)	1.13 ± 0.041 (0.99 - 1.33)	-0.025 ± 0.037 (-0.17 - 0.14)	-0.12,0.066	0.524	(0.88 - 1.32) [0.67,1.51]
Vitamin B_6 (mg/kg dwt)	7.16 ± 0.22 (6.57 - 8.06)	7.10 ± 0.22 (5.65 - 8.54)	0.063 ± 0.28 (-1.27 - 2.40)	-0.59,0.72	0.828	(4.93 - 7.24) [4.29,7.84]
Vitamin E (mg/kg dwt)	14.15 ± 1.70 (6.08 - 16.93)	14.07 ± 1.70 (1.74 - 17.77)	0.070 ± 1.46 (-11.15 - 14.39)	-2.93,3.07	0.962	(8.09 - 21.97) [0,29.69]

[a]dwt=dry weight; Vitamin B_1 =Thiamine; Vitamin B_2 =Riboflavin; Vitamin B_6 =Pyridoxine; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table H-7. Comparison of the Secondary Metabolites and Anti-nutrients Content in Grain from MON 88017 and Conventional Corn for Combined Field Sites

Component (Units)[a]	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.][b]
			Mean ± S.E. (Range)	95% CI (Lower, upper)	p-Value	
Ferulic acid (µg/g dwt)	2175.34 ± 46.31 (1986.75 - 2275.48)	2121.05 ± 46.31 (1927.55 - 2339.71)	54.29 ± 49.66 (-200.92 - 347.92)	-47.14,155.72	0.283	(1717.17 - 2687.57) [1415.19,3173.90]
p-Coumaric acid (µg/g dwt)	169.26 ± 7.26 (148.45 - 215.25)	154.83 ± 7.26 (141.41 - 173.24)	14.43 ± 9.88 (-14.72 - 72.55)	-9.75,38.61	0.194	(152.30 - 319.15) [43.13,384.34]
Phytic acid (% dwt)	0.95 ± 0.043 (0.83 - 1.05)	0.89 ± 0.043 (0.72 - 1.03)	0.058 ± 0.056 (-0.15 - 0.24)	-0.058,0.17	0.309	(0.45 - 1.00) [0.28,1.12]
Raffinose (% dwt)	0.17 ± 0.013 (0.14 - 0.20)	0.17 ± 0.013 (0.14 - 0.23)	0.00080 ± 0.0081 (-0.035 - 0.036)	-0.019,0.021	0.924	(0.073 - 0.22) [0,0.32]

[a]dwt=dry weight; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval
[b]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table H-8. Literature and Historical Ranges of Components of Corn Forage and Grain

Tissue/Component[1]	Literature Range[2]	Historical Range[3]
Forage		
Proximates (% dwt)		
Ash	2.43-9.64[a]; 2-6.6[b]	2.03-8.23
Carbohydrates	83.2-91.6[b]; 76.5-87.3[a]	80.6-90.8
Fat, total	0.35-3.62[b]; 1.42-4.57[a]	0.61-4.02
Moisture (% fw)	56.5-80.4[a];55.3-75.3[b]	42-78.8
Protein	4.98-11.56[a]	3.86-11.0
Fiber (% dwt)		
Acid detergent fiber (ADF)	18.3-41.0[b]; 17.5-38.3[a]	17.6-36.7
Neutral detergent fiber (NDF)	26.4-54.5[b]; 27.9-54.8[a]	29.6-55.2
Minerals (% dwt)		
Calcium	0.0969-0.3184[b]	0.0866-0.2754
Phosphorous	0.1367-0.2914[b]	0.1602-0.2914
Grain		
Proximates (% dwt)		
Ash	1.1-3.9[d]; 0.89-6.28[b]	0.81-3.09
Carbohydrates	77.4-87.2[b]; 82.2-88.1[a]	79.8-89.6
Fat, total	3.1-5.7[d]; 2.48-4.81[b]	1.74-4.83
Moisture (% fw)	7-23[d]; 8.18-26.2[b]	6.07-24.7
Protein	6-12[d]; 9.7-16.1[c]	6.15-14.8
Fiber (% dwt)		
Acid detergent fiber (ADF)	3.3-4.3[d]; 2.46-11.34[a,b]	2.3-9.33
Neutral detergent fiber (NDF)	8.3-11.9[d]; 7.58-15.91[b]	6.88-18.1
Total dietary fiber (TDF)	10.99-11.41[h]	-

[1]fw=fresh weight; dw=dry weight; Vitamin B_1 =Thiamine; Vitamin B_2 =Riboflavin; Vitamin B_6 =Pyridoxine.
[2]Literature range references: [a]Ridley et al., 2002. [b]Sidhu et al., 2000. [c]Jugenheimer, 1976. [d]Watson, 1987. [e]Watson, 1982. [f]Classen et al., 1990. [g]Dowd and Vega, 1996. [h]Choi et al., 1999.
[3] Historical range is from control samples analyzed in previous Monsanto Company studies.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table H-8 (cont). Literature and historical ranges of components of corn forage and grain

Tissue/Component[1]	Literature Range[2]	Historical Range[3]
Grain		
Minerals		
Calcium (% dwt)	0.01-0.1[d]	0.0024-0.0089
Copper (mg/kg dwt)	0.9-10[d]	0.98-3.43
Iron (mg/kg dwt)	1-100[d]	10.4-30.7
Magnesium (% dwt)	0.09-1[d]	0.082-0.16
Manganese (mg/kg dwt)	0.7-54[d]	3.2-9.89
Phosphorous (% dwt)	0.26-0.75[d]	0.24-0.46
Potassium (% dwt)	0.32-0.72[d]	0.29-0.53
Zinc (mg/kg dwt)	12-30[d]	14.1-37.2
Amino Acids	**(% total protein)**	**(% total amino acid)**
Alanine	6.4-9.9[e]	7.06-8.19
Arginine	2.9-5.9[e]	3.49-5.48
Aspartic acid	5.8-7.2[e]	5.97-7.36
Cystine	1.2-1.6[e]	1.61-2.94
Glutamic acid	12.4-19.6[e]	17.3-20.4
Glycine	2.6-4.7[e]	3.22-4.91
Histidine	2.0-2.8[e]	2.46-3.35
Isoleucine	2.6-4.0[e]	2.95-4.08
Leucine	7.8-15.2[e]	11.2-14.6
Lysine	2.0-3.8[e]	2.35-4.18
Methionine	1.0-2.1[e]	1.61-2.89
Phenylalanine	2.9-5.7[e]	4.6-5.76
Proline	6.6-10.3[e]	8.03-9.9
Serine	4.2-5.5[e]	3.45-5.63
Threonine	2.9-3.9[e]	2.87-4.01
Tryptophan	0.5-1.2[e]	0.39-1.04
Tyrosine	2.9-4.7[e]	1.93-4.32
Valine	2.1-5.2[e]	3.94-5.46

[1]fw=fresh weight; dw=dry weight; Vitamin B_1 =Thiamine; Vitamin B_2 =Riboflavin; Vitamin B_6 =Pyridoxine.
[2]Literature range references: [a]Ridley et al., 2002. [b]Sidhu et al., 2000. [c]Jugenheimer, 1976. [d]Watson, 1987. [e]Watson, 1982. [f]Classen et al., 1990. [g]Dowd and Vega, 1996. [h]Choi et al., 1999.
[3] Historical range is from control samples analyzed in previous Monsanto Company studies.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Table H-8 (cont). Literature and historical ranges of components of corn forage and grain

Tissue/Component[1]	Literature Range[2]	Historical Range[3]
Grain		
Fatty Acids	(% total fat)	(% total fatty acid)
16:0 Palmitic	7-19[e]	8.41-12.5
16:1 Palmitoleic	1[e]	0.05-0.18
18:0 Stearic	1-3[e]	1.33-2.61
18:1 Oleic	20-46[e]	20.1-37.7
18:2 Linoleic	35-70[e]	48.0-66.1
18:3 Linolenic	0.8-2[e]	0.74-1.45
20:0 Arachidic	0.1-2[e]	0.31-0.56
20:1 Eicosenoic	-	0.15-0.44
22:0 Behenic	-	0.075-0.3
Vitamins	**(mg/kg dwt)**	
Folic acid	0.3[d]	0.33-0.75 µg/g dwt
Niacin	9.3-70[d]	-
Vitamin B1	3-8.6[e]	0.2-0.33 mg/100g dwt
Vitamin B2	0.25-5.6[e]	0.83-1.74 µg/g dwt
Vitamin B6	5.3[d]; 9.6[e]	-
Vitamin E	3-12.1[e]; 17-47[d]	0.005-0.037 mg/g dwt

[1]fw=fresh weight; dw=dry weight; Vitamin B_1 =Thiamine; Vitamin B_2 =Riboflavin; Vitamin B_6 =Pyridoxine.
[2]Literature range references: [a]Ridley et al., 2002. [b]Sidhu et al., 2000. [c]Jugenheimer, 1976. [d]Watson, 1987. [e]Watson, 1982. [f]Classen et al., 1990. [g]Dowd and Vega, 1996. [h]Choi et al., 1999.
[3] Historical range is from control samples analyzed in previous Monsanto Company studies.

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw.

Appendix I: Appearance of Glyphosate-Resistant Weeds

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. The issue of glyphosate resistance is important to Monsanto because it can adversely impact the utility and life cycle of our products if it is not managed properly. The risk of weeds developing resistance and the potential impact of resistance on the usefulness of a herbicide vary greatly across different modes of action and are dependent on a combination of different factors. As leaders in the development and stewardship of glyphosate products for almost thirty years, Monsanto invests considerably in research to understand the proper uses and stewardship of the glyphosate molecule. This research includes an evaluation of some of the factors that can contribute to the development of weed resistance.

A. The Herbicide Glyphosate

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #: 1071-83-6), the active ingredient in the Roundup family of nonselective, foliar-applied, post-emergent agricultural herbicides, is among the world's most widely used herbicidal active ingredients. Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate kills plant cells by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Franz et al., 1997). This aromatic amino acid pathway is not present in mammalian metabolic systems (Cole, 1985). This mode of action contributes to the selective toxicity of glyphosate toward plants and to the low risk to human health from the use of glyphosate according to label directions. A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development, and does not bioaccumulate in mammals (Williams et al., 2000). Glyphosate has favorable environmental characteristics, including a low potential to move to through soil to reach ground water and its degradation over time by soil microbes. Because it binds tightly to soil, glyphosate's bioavailability is reduced immediately after use, which is why glyphosate has no residual soil activity. An ecotoxicological risk assessment concluded that the use of glyphosate does not pose an unreasonable risk of adverse effects to non-target species, such as birds and fish, when used according to label directions (Giesy et al., 2000).

B. Characteristics Related to Resistance

Today, some 172 herbicide-resistant species and 287 biotypes within those species have been identified (Heap, 2003). Most of them are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Le Baron, 1991; Shaner, 1995). Resistance usually has developed because of the long residual activity of these herbicides with the capacity to control weeds all year long and the selection pressure exerted by the repeated use of herbicides with a single target site and a specific mode of action. Using these criteria,

and based on current use data, glyphosate is considered to be a herbicide with a low risk for weed resistance (Benbrook, 1991). Nonetheless, a question has been raised as to whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide.

It is important to recognize that weed resistance is a herbicide-related issue, not a crop-related issue. The use of a specific herbicide with a herbicide tolerant crop is no different than the use of a selective herbicide over a conventional crop from a weed resistance standpoint. While the incidence of weed resistance is often associated with repeated applications of a herbicide product, its development depends very much on the specific herbicide chemistry in question as well as the plant's ability to inactivate them. Some herbicide products are much more prone to develop herbicide resistance than others. Glyphosate has been used extensively for three decades with very few cases of resistance development, particularly in relation to many other herbicides. A summary of some of those factors is described below.

B.1. Target Site Specificity

Target site alteration is a common resistance mechanism among many herbicide classes, such as acetolactate synthase-inhibitors and triazines, but is less likely for glyphosate.

An herbicide's mode of action is classified by the interference of a critical metabolic process in the plant by binding to a target protein and disrupting the required function. The "specificity" of this interaction is critical for the opportunity to develop target site mediated resistance. Because the herbicide contacts discreet amino acids during protein binding, changing one of these contact point amino acids can interrupt this binding. Specificity of inhibitor binding is dependent on the number and type of the amino acids serving as contact points and can be measured indirectly by counting the number of unique compounds that can bind in the same site. On one extreme, glyphosate is the only herbicide compound that can bind to EPSPS. Single amino acid substitutions near the active site have been observed for EPSPS, and while glyphosate binding is slightly weaker, these enzymes are also less fit. Similarly, high specificity is also observed for glutamine synthetase, binding three compounds including phosphinothricin in the active site (Crespo et al., 1999). Paraquat and diquat are the only two herbicides inhibiting photosystem I. No target site mutations have been reported to be responsible for resistance in these systems (Powles and Holtum, 1994).

On the other extreme are target enzymes that are efficiently inhibited by a wide array of compounds, e.g., acetolactate synthase (ALS) is inhibited by 53 and acetyl CoA carboxylase (ACCase) is inhibited by 21 separate herbicide compounds that bind both within and outside the active site (HRAC 2003; Tranel and Wright, 2002). These cases demonstrate that numerous non-critical amino acids are involved outside of the active site, offering a relatively large range of permissible mutations. In these two cases, a single amino acid change can result in virtual immunity to the class of herbicides and has directly led to the preponderance of weed species resistant to herbicides of these mode-of-action classes. Thus, 79 and 30 weed species have been found to be resistant to herbicides of the ALS and ACCase mode of action classes, respectively.

Glyphosate competes for the binding site of the second substrate, phosphoenolpyruvate in the active site of EPSPS and is a transition state inhibitor of the reaction (Steinrucken and Amrhein, 1984). This was recently verified by x-ray crystal structure (Schonbrunn et al., 2001). As a transition state inhibitor, glyphosate binds only to the key catalytic residues in the active site. Catalytic residues are critical for function and cannot be changed without a lethal or serious fitness penalty. Furthermore, very few selective changes can occur near the active site of the enzyme to alter the competitiveness of glyphosate without interfering with normal catalytic function. Therefore, target site resistance is highly unlikely for glyphosate. This was further illustrated in that laboratory selection for glyphosate resistance using whole plant or cell/tissue culture techniques were unsuccessful (Jander et al., 2003; Widholm et al., 2001; OECD, 1999).

B.2. Limited Metabolism in Plants

Metabolism of the herbicide active moiety is often a principle mechanism for the development of herbicide resistance. The lack of glyphosate metabolism or significantly slow glyphosate metabolism has been reported in several species and reviewed in various publications (Duke, 1988; Coupland, 1985). Therefore, this mechanism is unlikely to confer resistance to glyphosate in plants.

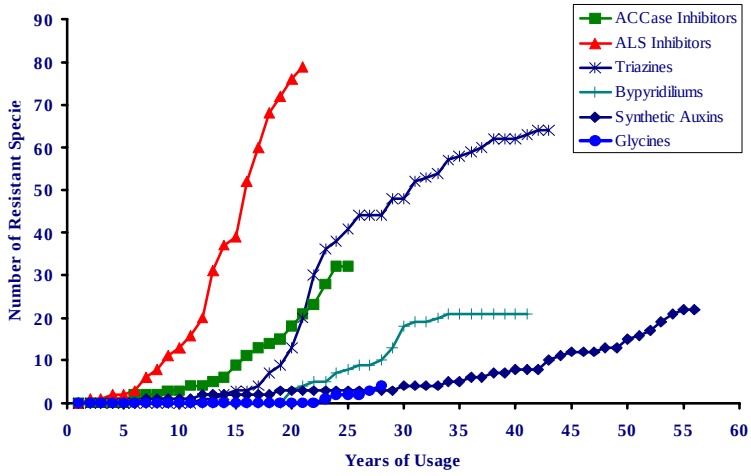
B.3. Lack of Soil Residual Activity

Herbicides with soil residual activity dissipate over time in the soil resulting in a sublethal exposure and in effect low dose selection pressure. Glyphosate adsorption to soils occurs rapidly, usually within one hour (Franz et al., 1997). Soil-bound glyphosate is unavailable to plant roots, so the impact of sublethal doses over time is eliminated. The postemerge-only activity of glyphosate allows for the use of a high dose weed management strategy.

The graph in Figure I-1 illustrates the instances of weed resistance to various herbicide families. The different slopes observed are largely due to the factors described above, which relate to chemistry and function, in addition to levels of exposure in the field. Glyphosate is a member of the glycine family of herbicides, which have experienced very limited cases of resistance despite almost three decades of use. The ALS inhibitors and triazine families, on the other hand, have experienced extensive cases of resistance even after they were available for only a relatively short period of time.

It is also important to recognize that each herbicide targets a large number of weeds, so the development of resistance in certain species does not mean the herbicide is no longer useful to the grower. For example, resistance of certain weeds to imidazolinone and sulfonyurea chemistries developed within three to five years after their introduction into cropping systems. Nevertheless, Pursuit (imidazolinone) herbicide had a 60% share of the U.S. soybean herbicide market despite the presence of a large number of resistant weeds because it was used in combination with other herbicides that controlled the resistant species. How weed resistance impacts the use of a particular herbicide varies greatly depending on the herbicide chemistry, the biology of the weed, availability of other control practices and the diligence with which it is managed.

Figure I-1. Number of Herbicide Resistant Weed Species Found By Years of Herbicide Family Use[1]



[1]Heap, 2003.

C. Weeds Resistant to Glyphosate

Weed resistance as generally defined as the naturally occurring inheritable ability of some weed biotypes within a given weed population to survive a herbicide treatment that should, under normal use conditions, effectively control that weed population. Thus, a resistant weed must demonstrate two criteria: 1) the ability to survive application rates of a herbicide product that once were effective in controlling it; and 2) the ability to pass the resistance trait to seeds. Procedures to confirm resistance generally require both field and greenhouse analyses, particularly if the level of resistance is relatively low. This correlation has been particularly important for the accurate detection of glyphosate resistance, for which the levels of resistance observed have been as low as 2X the susceptible biotypes.

Herbicide tolerance differs from resistance in that the species is not controlled but has the inherent ability to survive applications of the herbicide from the beginning. In other words, the species does not develop tolerance through selection but is innately tolerant.

As part of our product stewardship and customer service policy, Monsanto investigates cases of unsatisfactory weed control to determine the cause, as described in the performance evaluation program outlined in section E of this appendix. Weed control failures following application of Roundup agricultural herbicides are most often the result of management and/or environmental issues and are very rarely the result of herbicide resistance. The procedures included in Monsanto's performance evaluation program provide early detection of potential resistance, field and greenhouse protocols to

investigate suspected cases and mitigation procedures to respond to confirmed cases of glyphosate resistance.

To date, biotypes of only four weed species resistant to glyphosate have been identified and confirmed. In all cases, Monsanto worked with local scientists to identify alternative control options that have been effective in managing the resistant biotypes.

Lolium rigidum

In 1996 in Australia, it was reported that a biotype of annual ryegrass (*Lolium rigidum*) was surviving application of label recommended rates of glyphosate (Pratley et al., 1996). A collaboration was established with Charles Sturt University to develop an agronomic understanding of the biotype and investigate the mechanism of resistance. Where the biotype has been found, it has occurred within isolated patches within a field and does not appear to be widespread. The resistant biotype is easily controlled within conservation and conventional tillage systems with other herbicides, tillage or seed removal.

A large body of biochemical and molecular biology experiments between Australian ryegrass biotypes resistant and susceptible to glyphosate indicate that the observed resistance is due to a combination of factors. The mechanism of resistance appears to be multigenic and caused by a complex inheritance pattern, which is unlikely to occur across a wide range of other species. The mechanism is yet to be fully defined despite significant research effort; however, reduced cellular transport of glyphosate has been proposed (Lorraine-Colwill et al., 2003).

The resistant annual ryegrass biotype has also been observed in orchard systems of California and South Africa. Similar to the Australian locations, these fields are small and isolated. Monsanto established collaborations with local scientists to identify alternative control mechanisms, and the use of other herbicides, tillage, mowing, and seed removal have been very effective in controlling the ryegrass.

Annual ryegrass can occasionally be found in U.S. corn fields and can be controlled effectively with herbicides of several different classes such as primisulfuron (B), trifluralin (K), paraquat (D) and linuron (C2).

Lolium multiflorum

A population of Italian ryegrass (*Lolium multiflorum*) was reported to survive labeled rates of glyphosate by a scientist conducting greenhouse and field trials in Chile. Monsanto conducted field and greenhouse trials to confirm the resistance and worked with the researcher to identify alternative control options. A population was also identified in Brazil. The resistant biotypes have been found on only a few farms and are easily controlled through tank mixes with other herbicides and cultural practices.

Italian ryegrass is not commonly found in U.S. corn fields.

Eleusine indica
A population of *Eleusine indica (*goosegrass*)* was reported to survive labeled rates of glyphosate in some orchard systems in Malaysia. Monsanto entered into collaborations with the University of Malaysia and identified alternative control options to effectively manage the resistant biotype. Extensive molecular investigations determined that some of the resistant goosegrass plants have a modified EPSPS that is two to four times less sensitive to glyphosate than in more sensitive biotypes (Baerson et al., 2002). However, some resistant individuals did not exhibit the enzyme modification, suggesting that different mechanisms may be at play or resistance may be due to a combination of factors.

The resistant biotypes are easily controlled through application timing (applying glyphosate during the early growth stages), other herbicides, tillage and other cultural control practices.

Goosegrass can occasionally be found in U.S. cornfields and can be controlled effectively using a number of herbicide classes including sethoxydim (A), isoxaflutole (F2) and bromoxynil (C3).

Conyza canadensis
Laboratory and field investigations confirmed the presence of a glyphosate-resistant biotype of horseweed or marestail (*Conyza canadensis*) in certain states of the eastern and southern U.S. (VanGessel, 2001). The mechanism of resistance in the marestail biotype is currently under investigation. Findings thus far have been presented at regional and national weed science meetings and will be submitted for publication.

Investigations thus far indicate that this biotype has a heritable resistance ranging up to approximately six to eight times field herbicide application rates. Current data indicates that the heritance is dominant and transmitted by a singular nuclear gene. Resistance is not due to over-expression of EPSPS, glyphosate metabolism or reduction in glyphosate retention or uptake. Resistance is also not due to target site mutation, as the three isozymes of EPSPS identified in marestail were identical in sensitive and resistance lines. Our results demonstrate a strong correlation between impaired glyphosate translocation and resistance. Tissues from both sensitive and resistant biotypes showed elevated levels of shikimate, suggesting that EPSPS remained sensitive to glyphosate. Analysis of tissue shikimate levels relative to those of glyphosate demonstrated a reduced efficiency of EPSPS inhibition in the resistant biotypes. Our results are consistent with an exclusion mechanism for glyphosate resistance. Our current working hypothesis is that marestail resistance results from an alteration of glyphosate distribution that impairs its phloem loading and plastidic import.

The resistant marestail biotype has been observed in conventional and Roundup Ready cotton and soybean fields, and a stewardship program has been implemented that includes the use of a supplemental label, approved by EPA, to provide specific instructions on proper use of glyphosate herbicides in these counties where the resistant biotype has been confirmed. Growers in those counties are instructed to use specified

alternative control options, regardless of whether or not they had trouble controlling marestail on their farm the previous season, as a means to minimize spread of the resistant biotype. It has been recommended to growers in surrounding areas where the resistant biotype has not been confirmed that they use the alternative control options if marestail has been a difficult weed for them to control.

Alternative control options for use in corn are provided within the supplemental label for growers in that area. Specifically, growers are advised to start clean and apply a tank mixture of glyphosate with 2,4-D before marestail reaches 6" in height. Atrazine may also be included before emergence to provide residual control. In-crop recommendations include glyphosate plus Clarity or 2,4-D.

This stewardship program has proven effective in controlling the glyphosate-resistant biotype and minimizing its spread beyond the southern and eastern regions of the U.S.

Other Species

Populations of two weed species in South Africa, hairy fleabane (*Conyza bonariensis*) and buckhorn plantain (*Plantago lanceolata*), have been reported to be resistant to glyphosate (Heap, 2003). Monsanto is investigating the populations and has not confirmed resistance at this time. Various herbicides are available for control of these species, but they do not commonly occur in U.S. corn production.

Species that are tolerant to glyphosate, such as *Equisetum arvensis* (field horseweed), are occasionally described as "resistant." This characterization is technically inappropriate because glyphosate is not commercially effective on those weeds and they generally are not listed as controlled on Roundup agricultural herbicide product labels. Other species, such as *Convolvulus arvensis* (field bindweed) that are listed on the label may be partially tolerant or "difficult-to-control" with glyphosate alone. In these cases, additional herbicides are usually recommended to be tank-mixed with glyphosate. Still other species, such as *Abutilon theophrasti* (velvetleaf), may be listed as controlled by glyphosate on the label but a tank-mix recommendation for additional herbicide may be used in the field due to sensitive environmental or herbicide application conditions in certain counties or seasons.

In summary, Monsanto has effective product stewardship and customer service practices established to directly work with the grower communities and provide appropriate control measures for glyphosate-resistant weeds. Monsanto has collaborated with academic institutions to study these glyphosate-resistant biotypes and findings have been communicated to the scientific community through publications in peer-reviewed scientific journals and scientific meetings.

D. Weed management strategies for glyphosate

A key element of good weed management is using the correct rate of glyphosate at the appropriate window of application for the weed species and size present. Higher herbicide doses result in higher weed mortality and less diversity of resistance genes in

the surviving population (Matthews, 1994). Low herbicide rates also may allow both heterozygous and homozygous resistant individuals to survive (Maxwell and Mortimer 1994), further contributing to the build up of resistant alleles in a population. As resistance is dependent upon the accumulation of relatively weak genes, which it appears, may be the case for one or more of the four weed species that have evolved resistance to glyphosate, using a lethal dose of herbicide is critical.

Results that support these strategies are beginning to emerge from recent field research studies at several universities where it is documented that studies must be done in the field on the crop (Roush et al., 1990). Various weed management programs have been evaluated since 1998 to determine how they impact weed population dynamics. Studies were initiated in Colorado, Kansas, Nebraska, Wyoming (Wilson and Stahlman, 2003), and Wisconsin (Stoltenburg, 2002) to evaluate continuous use of Roundup Ready technology with exclusive use of glyphosate or inclusion of herbicides with other modes-of-action, and rotation away from Roundup Ready technology. These treatment regimes were compared to a conventional herbicide program for each crop evaluated. General observations after five years are:

1. Use of a continuous Roundup Ready cropping system with either glyphosate alone at labeled rates or incorporation of herbicides with other modes-of-action resulted in excellent weed control with no weed shifts or resistance reported.
2. Use of glyphosate at below labeled rates resulted in a weed shift to common lambsquarters at two locations (NE, WY).
3. In WI, ALS-resistant giant ragweed was selected for in the broad-spectrum residual herbicide regime implemented in the conventional corn cropping system. The continuous glyphosate system (using labeled rates) resulted in no significant weed shifts.

By using glyphosate at the recommended lethal dose, the build-up of weeds with greater inherent tolerance or any potential resistance alleles has been avoided over the duration of these studies. These results indicate that continuous Roundup Ready systems used over several years did not create weed shifts or resistant weeds when the correct rate of glyphosate was applied and good weed management was practiced.

E. Glyphosate stewardship program

Commercial experience, field trials and laboratory research demonstrate that one of the most important stewardship practices is achieving maximum control of the weeds. This can be accomplished by using the correct rate of glyphosate at the appropriate window of application for the weed species and size present, and using other tools or practices as necessary.

As the recognized leader in the development and commercialization of glyphosate, Monsanto is committed to the proper use and long-term effectiveness of glyphosate

through a four-part stewardship program: developing appropriate weed control recommendations; continuing research to refine and update recommendations; educating growers on the importance of good weed management practices; and responding to repeated weed control inquiries through a performance evaluation program.

E.1. Develop Local Weed Management Recommendations to Ensure Maximum Practical Control is Achieved

Weed control recommendations in product labels and informational materials are based on local needs to promote the use of the management tool(s) that are most appropriate technically and economically for each region. Furthermore, growers are instructed to apply the same principles when making weed control decisions for their own farm operation. Multiple agronomic factors, including weed spectrum and population size, application rate and timing, herbicide resistance status (where applicable) and an assessment of past and current farming practices used in the region or on the specific operation are considered to ensure appropriate recommendations for the use of glyphosate to provide effective weed control. Carefully developing and regularly updating the use recommendations for glyphosate are fundamental to Monsanto's stewardship program.

Weed Spectrum

Weed spectrum refers to all of the weed species present in a grower's field and the surrounding areas that may impact those fields. The spectrum may vary across regions, farm operations, and even among fields within a farm operation depending on environmental conditions and other factors. Weed control programs should be tailored on a case by case basis by identifying the target weeds present, considering the efficacy of glyphosate and other weed management tools against those particular weeds, and assessing if any are unlikely to be controlled sufficiently with glyphosate alone (not included on the Roundup brand agricultural herbicide label; difficult to control based on the agronomic and/or environmental conditions; or documented resistance to glyphosate). A formulation, rate, application parameters and additional control tools are recommended as necessary to optimize control of all weeds in that system.

Application Rate

Application rate is integral to the correct use of glyphosate and critical to obtain effective weed control. Significant research is conducted to identify the appropriate rate of glyphosate that should be applied for a particular weed at various growth stages in various agronomic and environmental conditions. These rates are included in rate tables provided in product labels and other materials. In addition, Monsanto recommends that growers use the rate necessary to target the most difficult to control weed in his system to minimize weed escapes. When recommending tank mixes, growers should consider the potential impacts on glyphosate efficacy through antagonism or below-recommended rates and make adjustments accordingly.

ApplicationTiming

Application timing is based on the growth stage of weeds, the size/biomass of weeds and the agronomic and environmental conditions at the time of application. Delaying the

application of glyphosate and allowing weeds to grow too large before applying the initial "recommended rate" of glyphosate will result in poor efficacy. Applying the glyphosate at a time while weeds are under agronomic stress (e.g., insect/disease) or environmental stress (e.g., moisture/cold) can also result in poor efficacy of control.

Compensating for a delayed application through subsequent applications may not be effective, as the first application may inhibit the growth of weeds and impair efficacy of the second application because the weeds may not be in an active growth process.

Correct application timing is dependent on the combined management of the weed spectrum, the size and layout of the farm operation and the feasibility to make timely applications of all weeds in the fields with labor and equipment available. Monsanto recommends an application timeline that targets susceptible growth stages of all weeds, and where applicable includes recommendations for inclusion of additional control tools as necessary to optimize control of all weeds on that farm.

Finally, it is important to assess the current agronomic practices used in that region or on that farm operation to integrate the glyphosate recommendations into the grower's preferred management system. Variables such as tillage methods, crop rotations, other herbicide programs, other agronomic practices, and the resistance status of the weeds to herbicides other than glyphosate can impact the spectrum of weeds present and the tools available to the grower.

Weed management recommendations communicated to the grower also incorporate other components of the glyphosate stewardship program including the use of certified seed, employing sanitary practices such as cleaning equipment between fields, and scouting fields and reporting instances of unsatisfactory weed control for follow up investigation.

E.2. Continuing Research

A fundamental component of Monsanto's leadership in glyphosate stewardship is continuing research on the recommended use of glyphosate and factors impacting its effectiveness. In addition to the extensive analyses conducted to determine the labeled rate of glyphosate prior to product registration, ongoing agronomic evaluations are conducted at the local level to refine weed management recommendations for specific weed species in specific locations.

Weed efficacy trials are part of ongoing efforts by Monsanto to tailor recommendations to fit local conditions and grower needs. Application rate and timing, additional control tools and other factors are included in these analyses. As a result of weed efficacy trials, changes are made to specific weed control recommendations where and when applicable, and modifications to local recommendations are highlighted to growers through informational sheets and other methods.

E.3. Education and Communication Efforts

Another key element of effective product stewardship and appropriate product use is education to ensure that growers understand and implement effective weed management plans and recommendations. Monsanto communicates weed management recommendations through multiple channels and materials to multiple audiences.

All internal technical and sales field representatives are required to take a weed management training course to understand the glyphosate stewardship program and the importance of proper product use. The training program is supported by ongoing weed management updates that highlight seasonal conditions and recommendations.

Monsanto weed management recommendations and the importance of sound agronomic practices are communicated to growers, dealers and retailers, academic extension and crop consultants through multiple tools:

a. Technology training programs; Highlighting weed management principles, weed management plans and practical management guidelines.
b. Technology use guide: Includes tables outlining appropriate rate and timing for different weed species and sizes.
c. Grower meetings: Conducted prior to planting to emphasize the importance of following local application recommendations.
d. Marketing programs: Designed to reinforce and encourage the continued adoption and use of weed management recommendations by the grower (e.g., recommended rate and timing of application, additional weed control tools when applicable).
e. Informational Sheets: Issued to growers and dealers/retailers to highlight local recommendations for specific weeds.

As with most stewardship efforts, education is key to help growers and other stakeholders understand the importance of proper product use and encourage those practices in the field.

E.4. Performance Inquiry Evaluation and Weed Resistance Management Plan

To support and enhance Monsanto's weed management principles and recommendations, Monsanto implements a performance evaluation program based on grower performance inquiries and field trial observations. The goal of the program is to continue to adapt, modify and improve Monsanto's weed control recommendations, with a focus on:

a. Particular weeds and growing conditions;
b. Providing product support to customers who are not satisfied with their level of weed control; and
c. Identifying and investigating potential cases of glyphosate resistance early so that mitigation strategies can be implemented.

The grower generally reports instances of unsatisfactory weed control following glyphosate application to Monsanto or the retailer. It is important to Monsanto, as part of its customer service and stewardship commitment, that these product performance inquiries are acted upon immediately, resolved to the satisfaction of the customer and not repeated.

The vast majority of inquiries is due to application error or environmental conditions and resolved through a phone conversation with the grower. However, a system is in place to investigate a repeated performance inquiry for a specific weed on a specific field within the same year. The investigation considers the various factors that could account for ineffective weed control such as:

a. Application rate and timing;
b. Weed size and growth stage;
c. Environmental and agronomic conditions at time of application;
d. Herbicide application calibration

In all cases, the first priority is to provide control options to the grower so that satisfactory weed control is achieved for that growing season. The majority of repeated product performance inquiries is due to improper application or environmental/agronomic conditions and not repeated. However, if the problem occurs again in that field and does not appear to be due to application or growing condition factors, then steps are taken to determine if resistance is the cause as outlined in the Monsanto Weed Resistance Management Plan.

The Monsanto Weed Resistance Management Strategy consists of three elements:

a. Identification process for potential cases of glyphosate resistance;
b. Initiation of steps to respond to cases of suspected resistance; and
c. Development and communication of guidelines to incorporate resistance mitigation into weed management recommendations.

Identification of potential cases of glyphosate resistance is accomplished through evaluation of product performance inquiries and local field trials. These efforts provide an early indication of ineffective weed control that may indicate potential resistance.

If the follow up investigation clearly indicates that the observation is due to application error or agronomic/environmental conditions, then appropriate control options are recommended to the grower for that season and the grower receives increased education on the importance of proper product use. The vast majority of weed control inquiries fall into this category.

If repeated lack of control is observed and does not appear to be due to application error or environmental conditions, then a field investigation is conducted by Monsanto to analyze control of the weed more thoroughly.

The vast majority of field investigations do not repeat the insufficient control reported by the grower, largely due to characteristics of the mode of action of glyphosate that make subsequent applications by the grower ineffective. The weed usually must be in an active growth phase in order for glyphosate to be effective, application error or environmental conditions that result in insufficient glyphosate to kill the weed often stunt its growth such that subsequent applications by the grower are ineffective. Monsanto's field investigations at this stage remove that artifact by ensuring that the weeds tested are in an active growth phase. If the field investigation confirms that agronomic factors account for the observation, then the grower receives increased education on proper application recommendations.

In addition, the internal network of Monsanto technical managers and sales representatives in the surrounding area are notified to highlight any problematic environmental conditions or application practices that may be common in that area. Critical information regarding location, weed species, weed size, rate used and the potential reason for lack of control are captured, and the results are reviewed annually by the appropriate technical manager to identify any trends or learnings that need to be incorporated into the weed management recommendations.

If the reported observation is repeated in the field investigation, then a detailed performance inquiry is conducted and greenhouse trials are initiated. If greenhouse trials do not repeat the observation and the weed is clearly controlled at label rates, then a thorough follow up visit is conducted with the grower to review the application recommendations and conditions of his operation that may be impacting weed control. The internal network of agronomic managers is notified of the results to raise awareness of performance inquiries on that weed the following season. If the greenhouse efficacy trials do indicate insufficient control at label rates, then detailed studies are conducted to determine if the weed is resistant.

Resistance is considered to be confirmed if the two criteria outlined in the Weed Science Society of America definition of resistance are deemed to be fulfilled either through greenhouse data or experience with similar cases:

1) The suspect plant is demonstrated to tolerate labeled rates of glyphosate that previously were effective in controlling it; and
2) The suspect plant is capable of passing that ability to offspring (the trait is heritable).

Additional field trials will be initiated simultaneously as these investigations are conducted to identify the most effective and efficient alternative control options for that weed in various growing conditions. The research may be conducted internally as well as through collaboration with external researchers.

If resistance is confirmed, then the scientific and grower communities are notified as appropriate and a weed resistance mitigation plan is implemented. The mitigation plan is designed to manage the resistant biotype through effective and economical weed

management recommendations implemented by the grower. The scope and level of intensity of the mitigation plan vary depending on a combination of the following factors:

- biology and field characteristics of the weed (seed shed, seed dormancy, etc.),
- importance of the weed in the agricultural system,
- resistance status of the weed to other herbicides with alternate modes of action, and
- availability of alternative control options,

These factors are analyzed in combination with economic and practical management considerations to develop a tailored mitigation strategy that is technically appropriate for the particular weed and incorporates practical management strategies that can be implemented by the grower.

Once developed, the mitigation plan is communicated to the grower community through the use of supplemental labeling, informational fact sheets, retailer training programs, agriculture media or other means as appropriate.

The final step of the Weed Resistance Management Plan may include extensive genetic, biochemical or physiological analyses of confirmed cases of glyphosate resistance in order to elucidate the mechanism of resistance. Findings of this research are communicated to the scientific community through scientific meetings and publications, and information pertinent to field applications is incorporated into weed management recommendations.

F. Summary

Development of weed resistance is a complex process that is very difficult to accurately predict, and no single agronomic practice will mitigate resistance for all herbicides or all weeds. As a result, weed resistance needs to be managed on a case-by-case basis and tailored for the particular herbicide and grower needs. Using good weed management principles built upon achieving high levels of control through proper application rate, choice of cultural practices and appropriate companion weed control tools will allow glyphosate to continue to be used effectively.

The key principles for effective stewardship of glyphosate use, including Roundup Ready crops, include: 1) basing recommendations on local needs and using the tools necessary to optimize weed control; 2) proper rate and timing of application; and 3) responding rapidly to instances of unsatisfactory weed control.

Supplemental Section. Copies of Reports Submitted to the FDA and EPA [CBI-deleted]

Copies of the following reports submitted to the FDA and EPA (Supplements 1-5), which are claimed as Confidential Business Information (CBI), are not provided with this petition.

FDA

Supp. 1. Bogdanova, N.N. 2004. Food and Feed Safety and Nutritional Assessment of MON 88017 Corn (BNF 0097). Prepared by Monsanto Company and submitted to the FDA on March 30, 2004.

EPA

The following reports were submitted to the EPA on Jan 22, 2004, as part of the registration application for MON 88017.

Supp. 2. Sidhu, R.S. 2004. Human Health and Environmental Assessment of the Plant-Incorporated Protectant *Bacillus thuringiensis* Cry3Bb1 Protein Produced in MON 88017. MSL-18835, an unpublished report prepared by Monsanto Company.

Supp. 3. Beasley, K.A., H.M. Anderson, P.B. Wimberley, D.W. Mittanck, and R.P. Lirette. 2002. Molecular Analysis of YieldGard® Rootworm/Roundup Ready® Corn Event MON 88017. MSL-17609, an unpublished study conducted by Monsanto Company.

Supp. 4. Bhakta, N.S., A.J. Hartmann, and J.C. Jennings. 2003. Cry3Bb1 and CP4 EPSPS Protein Levels in Corn Tissues Collected from MON 88017 Corn Produced in U.S. Field Trials Conducted in 2002. MSL-18823, an unpublished study conducted by Monsanto Company.

Supp. 5. Duan, J.J., M.S. Paradise, and C. Jiang. 2003. Evaluation of Functional Equivalence of Two Cry3Bb1 Protein Variants Against Susceptible Coleopteran Species. MSL-18799, an unpublished study conducted by Monsanto Company.

[Supplements 1-5 are CBI-deleted]