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MONSANTO

Study Title

Public Interest Assessment Supporting Registration of *Bacillus thuringiensis* Cry3Bb1 Protein and the Genetic Material (Vector ZMIR13L) Necessary for its Production in Corn Event MON 863

Data Requirement

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Author

Dennis P. Ward, Ph.D.

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Submittor

Monsanto Company
600 13th Street N.W., Suite 660
Washington, DC 20005

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Statement of No Data Confidentiality Claim

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COMPANY: Monsanto Company

COMPANY AGENT: ________________________

Dennis P. Ward, Ph.D.
Regulatory Affairs Manager

DATE: April 9, 2002

GLP Compliance Statement

This assessment was not conducted under the Good Laboratory Practice standards as set forth in 40 CFR Part 160.

Submitter: Dennis P. Ward
Regulatory Affairs Manager

Date: April 9, 2002

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Key to Abbreviations

ac	Acre
a.i.	Active ingredient
B.t.	*Bacillus thuringiensis*
bu	Bushel
CRW	Corn rootworm (*Diabrotica* spp.)
cry3Bb1	DNA sequence that encodes the protein Cry3Bb1
Cry3Bb1	Engineered variant of the wild type protein that is selectively toxic to Coleoptera species
DNA	Deoxyribonucleic acid
EPA	United States Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act of 1996
FR	United States Federal Register
IPM	Integrated pest management
LOC	Level of concern
MON 853	Monsanto code assigned to a corn event transformed with vector ZMIR14L
MON 863	Monsanto code assigned to a corn event transformed with vector ZMIR13L
NASS	National Agricultural Statistics Service
NCGA	National Corn Growers Association
RDR	Root damage rating (Iowa State University)
RQ	Risk quotient
USDA	United States Department of Agriculture

I. Summary

Corn (*Zea mays* L.) is the largest crop grown in the United States in terms of acreage planted and net value. In 2000, corn production covered 79.5 million acres, yielding 10 billion bushels with a net value of $18.4 billion (NCGA, 2001). Corn yields are negatively impacted by a number of insect pests. One of the most pernicious in the U.S. Corn Belt is the corn rootworm (*Diabrotica* spp.). 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. Approximately 14 million acres of corn were treated with organophosphate, carbamate or pyrethroid insecticides to limit corn rootworm damage in year 2000 (Doane, 2001). Corn rootworm has been described as the billion dollar pest complex based on costs associated with the purchase of soil insecticides and crop losses due to pest damage (Metcalf, 1986).

Monsanto Company has developed, through the use of recombinant DNA techniques, corn plants that are protected from damage due to feeding by corn rootworm larvae. The tissues of these plants produce a modified *Bacillus thuringiensis* (subspecies *kumamotoensis*) Cry3Bb1 protein that is selectively toxic to corn rootworm species. A synthetic variant of the *cry3Bb1* gene was prepared by Monsanto and incorporated into transformation vector, ZMIR13L. Transformation of plants with this vector resulted in the production of corn event MON 863. Cry3Bb1 protein and genetic material necessary for its production in corn event MON 863 are the subject of an application for a FIFRA Section 3 registration.

The benefits offered by commercialization of this technology are substantial. Corn hybrids containing event MON 863 are consistently more efficacious than soil-applied insecticides in protecting roots from larval feeding damage. This superior performance is expected to result in a significant yield advantage for growers planting MON 863 hybrids. Preliminary estimates place this yield benefit at 1.5 to 4.5%. For a reasonable range of prices and yields, the value of this yield benefit to growers is $4-$12/ac relative to the use of a soil-applied insecticide, depending on corn rootworm pressure.

Cry3Bb1 is far less hazardous than all insecticide active ingredients currently registered for corn rootworm control. The majority of corn rootworm insecticides are classified as restricted use and virtually all are undergoing reassessment by EPA due to significant environmental risks. The adoption of MON 863 hybrids provides an opportunity to tremendously reduce the occupational and environmental risks currently associated with the manufacture, transportation, storage, handling, application and disposal of conventional insecticides. In excess of seven million pounds of insecticide active ingredient are annually applied to 14 million acres of corn for the control of corn rootworms. At product maturity, MON 863 hybrids have the potential to reduce these applications by millions of pounds. Registration of plant-incorporated Cry3Bb1 to limit crop losses caused by corn rootworms will provide substantial benefits for growers and a reasonable certainty of no harm for humans and the environment.

II. Introduction

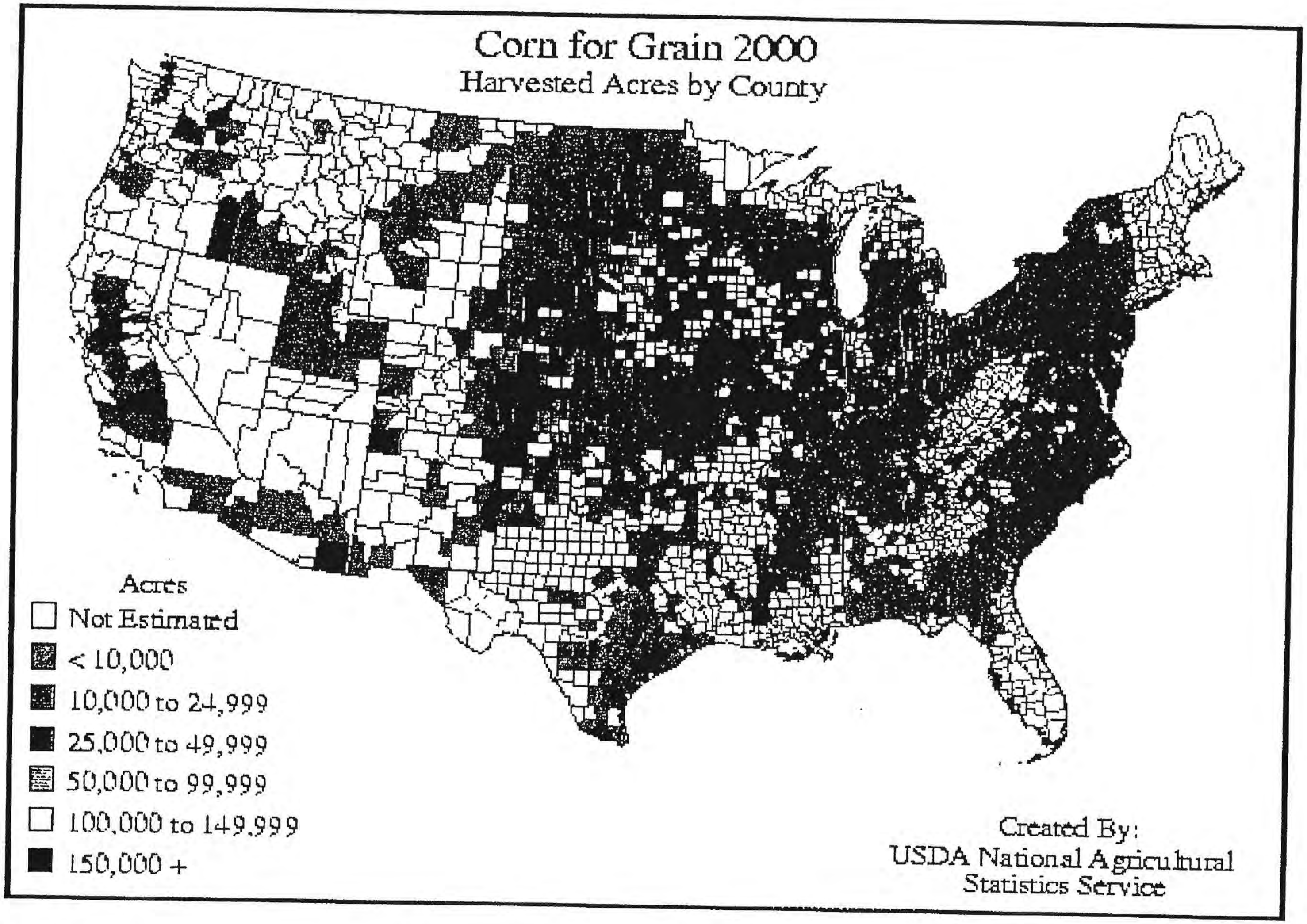
Corn is the major cultivated crop in the U.S., with total production exceeding half of that for the entire world. In 2000, U.S. corn production covered 79.5 million acres, yielding 10 billion bushels with a net value of $18.4 billion (NCGA, 2001). U.S., exportation of corn grain totaled $2.1 billion. Approximately 80% of harvested corn is consumed as livestock, poultry and fish feed. Fermentation to ethanol for use as a fuel supplement consumed 0.5 billion bushels of the 2000 harvest; use of grain for this purpose is expected to grow dramatically in coming years (NCGA, 2001). The remainder of harvested grain is processed into a wide variety of higher value food and industrial products (*e.g.*, cereal, oils, sweeteners, starch, adhesives, *etc.*).

Figure 1 provides an illustration of the regional distribution of harvested corn acres in the continental U.S. for year 2000. A major share of corn production is concentrated in seven north central Corn Belt states: Illinois, Indiana, Iowa, Minnesota, Nebraska, Ohio and Wisconsin. This region has a combination of ideal weather and soil conditions for growth (Olson and Sander, 1988).

Corn is an annual plant; the duration of its life cycle depends on the cultivar and the environment in which the cultivar is grown (Hanway, 1966). The bulk of corn is produced between latitudes 30° and 47 (Shaw, 1988). Practically no corn is grown where the mean midsummer temperature is <19°C or where the average nighttime temperature during the summer months falls much below 13°C. The greatest production occurs where the warmest month isotherms range between 21 and 27 C and the freeze-free season lasts 120 to 180 days. Corn is grown in areas where annual precipitation ranges from 25 to >500 cm. Summer rainfall of 15 cm is approximately the lower limit for corn production without irrigation.

Climate has a significant impact on corn production (Shaw, 1988). The influence of weather on corn starts even before planting. Conditions before planting are especially important in determining soil moisture reserves. Planting to emergence depends on soil temperature, soil moisture, soil aeration and seed vigor. Before germination, the seed absorbs water and swells. With warmer temperatures, less water needs to be absorbed so that germination will start earlier and proceed faster at higher temperatures, assuming water is available. The time to crop emergence varies with environmental conditions and, to a lesser degree, with planting depth, while development is affected directly by soil temperature and indirectly by air temperature. Weather is a major factor in determining planting time. Relatively early planting in the U.S. generally results in higher yields than late planting. During the early stages of plant development the corn plant requires a limited amount of moisture for the small growth that takes place. Growth during the early vegetative stage is strongly related to soil temperature. Growth during later stages of vegetative development is more closely related to air temperature and rainfall. The time at which tasseling and silking occur are also weather dependent. During the ear-filling stage, significant reduction in yield can occur from moisture stress or extremely high temperatures. An early freeze before physiological maturity may cause serious yield losses, especially if the corn is a late maturing variety. Overall, crop genetics, climate and soil conditions are major determinants of crop yield.

Figure 1. Distribution of acres harvested for corn grain by county in the continental U.S. for year 2000.



Farmers have hundreds of corn hybrids from which to choose. Available hybrids differ widely in agronomic characteristics, including length of growing period. Adjustments in cropping systems, as well as planting practices (*e.g.*, tillage and row spacing), impact crop yields. Optimizing soil nutrients is necessary for achieving maximum yield potential.

Yield losses due to weeds, diseases and insects were huge until the introduction of crop protection chemicals in the 1960s (Olsen and Sander, 1988). Weeds compete with crops for light, nutrients, water and other growth factors. If weeds are left uncontrolled, corn simply cannot be grown successfully (Shaw, 1988). Estimates of corn yield loss caused by pathogens have ranged from 2 to 17% (Smith and White, 1988). In addition, a corn crop is susceptible to attack by a variety of insects from the time it is planted until it is consumed as food or feed (Dicke and Guthrie, 1988). The most economically significant insect pests of corn are: the European corn borer [*Ostrinia nubilalis* (Hübner)], the American sugarcane borer (*Diatraea saccharalis*), the southwestern corn borer (*D. grandiosella*), the lesser cornstalk borer [*Elasmopalpus lignosellus* (Zeller)], *Heliothis* spp., the fall armyworm [*Spodoptera frugiperda* (J. E. Smith)], and the corn rootworm complex (*Diabrotica* spp.). Pests of secondary economic importance in corn include both soil-dwelling insects that feed on roots or other subterranean tissue (*e.g.*, wireworms, billbugs, webworms, white grubs, corn root aphids, the seed corn maggot, grape colaspis and seedcorn beetles) and aboveground insects that attack the stalk, leaf and ear (*e.g.*, cutworms, corn leaf aphids, chinch bugs, grasshoppers, corn flea beetles and Japanese beetles).

In addition to direct damage caused by feeding on plant tissue, insects play an important role in the transmission or dissemination of pathogenic organisms during corn development. Soil abounds in microorganisms, particularly fungi, which may infect plant parts injured by soil-dwelling insects. In much of the Corn Belt, pathogenic fungi probably pose more problems in corn production than any other group of organisms. Primary roots of the seedling and the radical and seminal roots are commonly infected with *Fusarium* spp. after they have served their function and become senescent. Feeding by *Diabrotica* rootworms has been associated with increased frequencies of *Fusarium* infection (Dicke and Guthrie, 1988); rootworm feeding may also lead to increased incidences of stalk rots. These pathogenic infections can lead to reduced crop quality, harvestability and yield.

III. The Corn Rootworm Pest

Corn yields are adversely impacted by a number of insect pests. One of the most pernicious pests in the U.S. Corn Belt is the corn rootworm complex (*Diabrotica*, spp.), comprised primarily of the northern corn rootworm (*D. barberi*, Smith and Lawrence), the western corn rootworm (*D. virgifera virgifera*, LeConte) and the Mexican corn rootworm (*D. virgifera zeae*, Krysan and Smith); refer to Figure 2. One additional *Diabrotica* specie, the southern corn rootworm (*D. undecimpunctata howardi*, Barber), inhabits the southeastern coastal regions of the U.S. and is a relatively minor pest of corn.

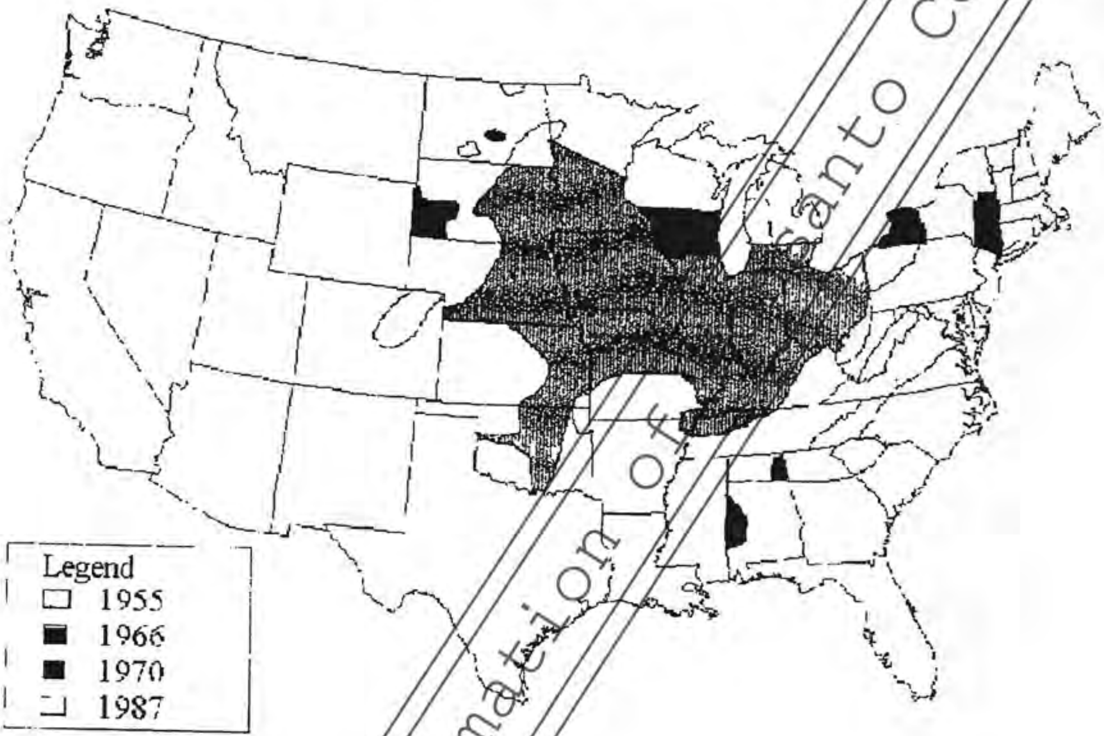
Western, northern, and Mexican corn rootworms have a similar life cycle. Each evolves through a single generation per year, and corn is the only economically important host. Oviposition commences in July with eggs being buried to a depth of two to four inches near the base of corn plants. The eggs over-winter, and the onset of hatch ranges from May to mid-June of the following season. Temperature and moisture vary with soil depth, both impact over-wintering survival and time of hatch. After hatching rootworm larvae (Figure 3) feed on corn roots for three to four weeks, passing through three instar growth stages. The mature larvae pupate in earthen cells and eclosion takes place from mid-June to early July. Adults live for 75 to 85 days, feeding on corn foliage, pollen and silks. Eggs are laid in the late summer and early fall (O'Day *et al.*, 1998).

Figure 2. Corn rootworm adults: western (top), northern (left) and southern (right).

Figure 3. Corn rootworm larvae.

Corn rootworms are widely distributed throughout the corn growing regions in the U.S. that are east of the Rocky Mountains (refer to Figures 4, 5 and 7). The pest is also found in Mexico, Brazil, and many countries of Europe. The levels of larval infestation are highest in in fields that are cropped continuously with corn (*i.e.* continuous corn). Feeding by western, northern, and Mexican corn rootworm larvae is generally greatest on roots near the soil surface; when these are consumed, the next lower node is attacked. First and second instars leave brown feeding scars or they tunnel from root tips to the plant base, destroying root hairs and small roots. Third instars cause the majority of root damage, generally feeding on the larger primary roots near the stalk and the first set of brace roots. Root feeding causes physiological stress that stunts plant growth and can lead to plant lodging (*i.e.*, falling over). Physiological stress and lodging can also have an adverse impact on crop yield.

Figure 4. Distribution and spread of the northern corn rootworm in the continental U.S.; the different colored regions represent the extent and pace of its migration across the Corn Belt (modified from Chiang, 1973, and Krysan and Smith, 1987).



The first indication of rootworm injury to corn may be in late June or early July when plants fall over after strong winds and heavy rainfall. Yield losses depend on the extent of larval feeding and on plant maturity, soil fertility, soil moisture at peak injury, and ability of the hybrid to regenerate root tissue. Drought stress worsens the effects of root pruning. Yield loss can result from root pruning and tunneling that disrupt the transport of nutrients and water from the root system (Reidell, 1990; Spike and Tollefson, 1991). The lack of root support can lead to gooseneck lodging (see Figure 6), which complicates harvesting, and root feeding can result in the invasion of plant tissue by secondary pathogens such as bacteria and fungi, increasing the incidence of root rots. Adult feeding on the aboveground portions of the plant rarely impacts crop yield.

Figure 5. Distribution and spread of the western corn rootworm in the continental U.S.: the different colored regions represent the extent and pace of its migration across the Corn Belt (modified from Chiang, 1973, and Krysan and Smith, 1987).

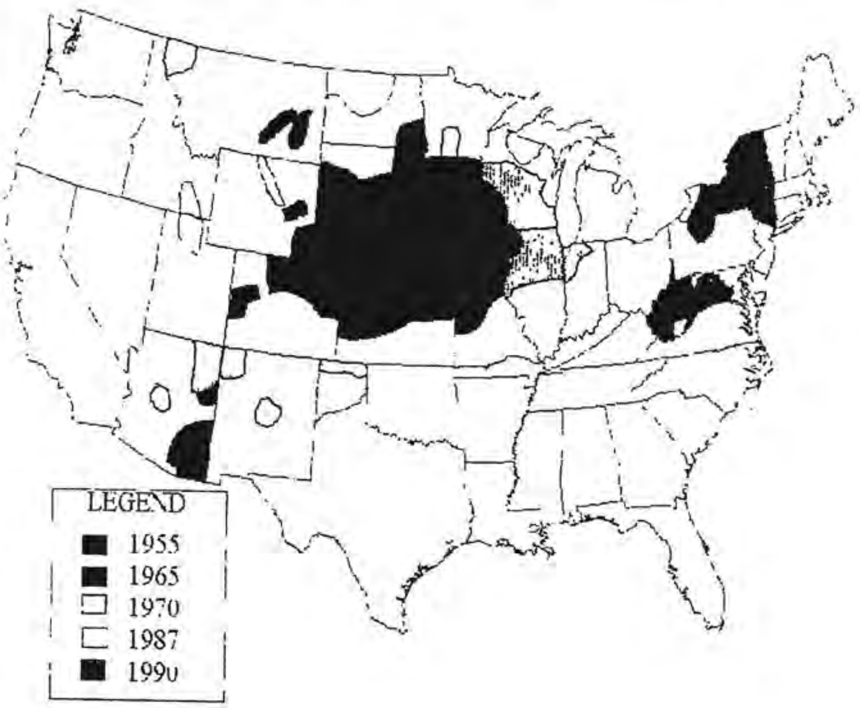


Figure 6. Goosenecked corn.

A scoring system developed by Hills and Peters (1971) has been widely used for rating root damage caused by corn rootworm larval feeding. This scoring system is commonly referred to as the Iowa State University root rating scale. Corn plants are dug and the roots are rated on a scale of 1-6. Table 1 describes the level of damage associated with each rating. A photographic representation of root damage ratings is displayed in Figure 8.

Figure 7. Distribution of the Mexican corn rootworm in the southern U.S. and Mexico.

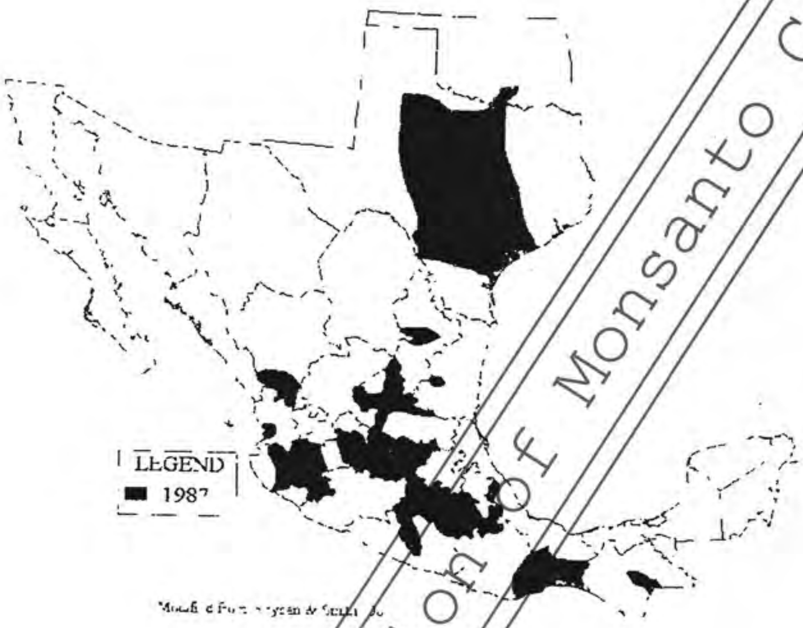
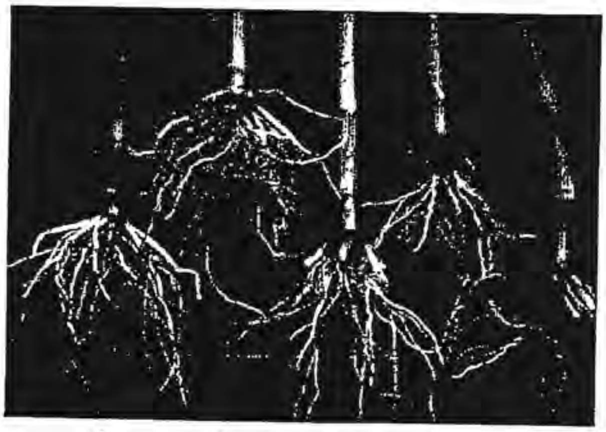


Table 1. Description of the Iowa State University 1 to 6 root damage rating (RDR) scale for assessing corn rootworm larval feeding damage.

RDR	Extent of Larval Root Feeding
1 -	No damage or only a few minor feeding scars
2 -	Feeding scars evident, but no roots pruned to within 1.5" of stalk
3 -	One or more roots pruned to within 1.5" of stalk, but never an entire node destroyed
4 -	One node of roots (or equivalent) destroyed
5 -	Two nodes of roots (or equivalent) destroyed
6 -	Three or more nodes of roots destroyed

Figure 8. Corn roots rated for damage caused by rootworm larval feeding using the Iowa 1 to 6 rating scale.



2 3 4 5 6

Increasing Damage

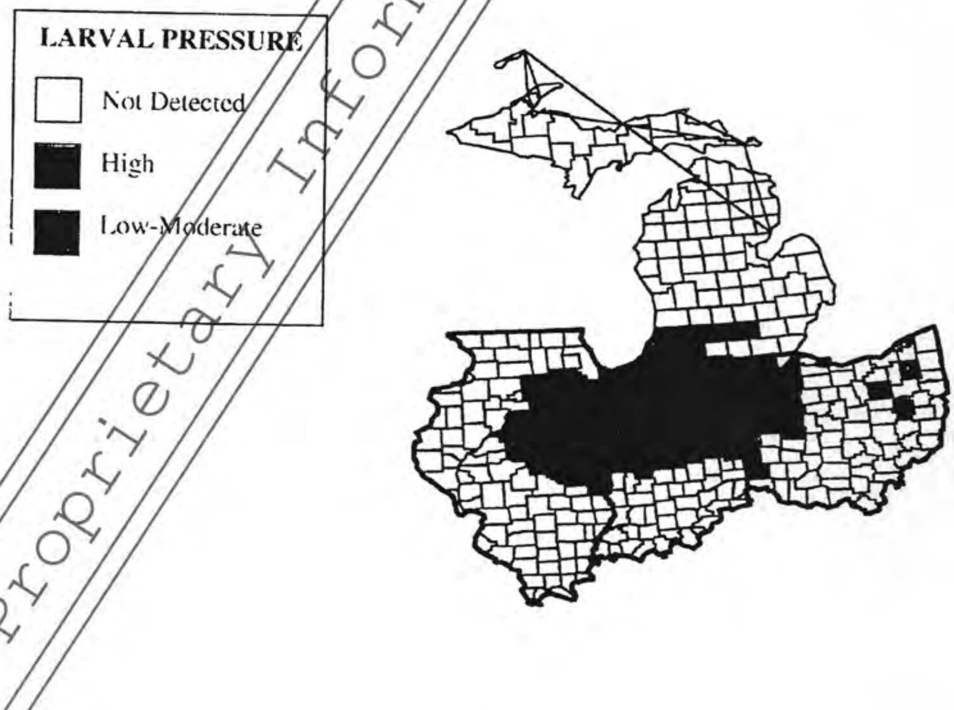
Root damage ratings are inversely correlated with crop yield. Early regression estimates predicted a reduction of 5.83 bu/ac in yield for every unit increase in root damage rating. However, yield losses can be much higher. Larval feeding has the potential to cause yield losses ranging from 10.7 to 85.8 bu/ac if left untreated (Apple *et al.*, 1977). A root damage rating as low as 2.5 is generally considered to be an economic threshold for growers (Steffey *et al.*, 1999). This represents the estimated root rating at which yield loss caused by larval feeding is equal to or greater than the cost of applying an insecticide.

Growers mitigate corn rootworm damage primarily through crop rotation or the use of soil insecticides. Historically, crop rotation has provided highly effective protection from corn rootworm damage in many agronomic situations because it breaks the pest's life cycle. However, several factors now limit the usefulness of this management strategy. First, many growers prefer the option of continuous (*i.e.*, nonrotated) corn production, even if this practice requires increased chemical inputs for soil fertility and insect control. Second, researchers have confirmed the existence of a northern corn rootworm variant in Iowa, Minnesota, Nebraska and South Dakota that exhibits an extended diapause period (see Figure 9). The eggs of this variant are able to survive through the noncorn years of crop rotation to yield larvae that affect first year corn (Ostlie, 1987; Tollefson, 1988; Gray *et al.*, 1998). Third, and of critical importance, crop rotation is no longer effective as a cultural corn rootworm management option in east central Illinois and northern Indiana due to the rapid spread of a new strain of western corn rootworm that, unlike previous populations, lays its eggs in soybean fields (Onstad and Joselyn, 1999; O'Neal *et al.*, 1999). The eggs of this western corn rootworm variant overwinter in soybean fields and emerge the following year in corn. Based on the rapid expansion of this variant population since its initial discovery in Illinois, it is expected to continue to spread throughout the Corn Belt (see Figure 10).

Each of these factors has increased grower reliance on chemical insecticides for corn rootworm control. The most common insecticide regime is the application of a granular soil

Figure 9. Geographic distribution of the northern corn rootworm extended diapause variant (shaded area) in Iowa, Minnesota, Nebraska and South Dakota.

Figure 10. Distribution the western corn rootworm soybean variant (shaded area) throughout Illinois, Indiana, Michigan and Ohio.



insecticide, either in-furrow or banded, at the time of planting. In some agronomic situations, post-emergence insecticide sprays are applied for adult suppression. The National Agricultural Statistics Service (NASS) of the U.S. Department of Agriculture (USDA) has compiled statistics on year 2000 corn insecticide use across 18 states comprising 73.8 million planted acres of corn (NASS, 2001). These statistics indicate that 9.8 million pounds of insecticide active ingredients registered for corn rootworm control were applied on more than 31% of the planted acres. Continued spread of the extended diapause and soybean variants of the corn rootworm has increased the number of first year corn acres needing an insecticide treatment. These data from NASS are in close agreement with and complement findings on insecticide usage from The 2000 AgroTrak™ Study conducted by Doane Marketing Research (2001).

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IV. Corn Insecticide Market

Management protocols for limiting economic loss caused by corn rootworm have relied on crop rotation or the use of chemical insecticides. Crop rotation has historically been the primary rootworm control measure. However, grower preference for continuous corn production in many situations, along with the recent changes in rootworm populations, has increased grower reliance on chemical control options. In typical agronomic situations, pest management guidelines recommend late summer scouting of fields for adult beetles and the use of local economic thresholds to determine the need for an insecticide application the following season. Many growers do not scout, preferring to treat continuous corn acres with a soil insecticide at planting, as has been recommended in Iowa (Foster *et al.*, 1986). Granular, and to a lesser extent liquid, insecticide formulations are applied in-furrow or banded at the time of planting. Insecticides may also be applied at cultivation. In some areas of the western Corn Belt, adult suppression is employed through overhead insecticide application timed to disrupt oviposition. Other approaches for control of corn rootworms, such as biological or pheromone control measures and fertility management, have not proved to be viable options for most large scale farming operations (Metcalfe and Lampman, 1997; Jackson, 1996). Conventional corn hybrids offering effective host plant resistance to corn rootworms have not been developed (Gray and Steffey, 1998a).

Numerous insecticide active ingredients in granular and liquid formulations are registered by EPA for control of corn rootworm larvae and adults. A list of these products is contained in Table 2. The active ingredients contained in these products are members of the organophosphate (9), synthetic pyrethroid (6), carbamate (3) and phenyl pyrazole (1) classes of chemistry. All display a broad spectrum of activity and operate via a neurotoxic mechanism of action. The majority (68%) of these products are classified 'Restricted Use' by EPA and can only be used by certified applicators. The labels of all these products carry warnings about significant hazards to the environment. Most are highly toxic to fish, birds and other wildlife species. Each year there are confirmed reports of human illness, as well as fish and bird poisonings, associated with the use of these products. Furthermore, there is evidence suggesting that some insecticides may be losing their effectiveness for controlling corn rootworm infestations. Resistance to some adult control insecticides (*e.g.*, methyl parathion and carbaryl) has been reported (Meinke *et al.*, 1997 and 1998).

Six years ago Congress passed the Food Quality Protection Act of 1996 (FQPA). This legislation significantly amended the Federal Insecticide Rodenticide and Fungicide Act and the Federal Food, Drug and Cosmetic Act. This statute required that EPA establish a single, health-based standard for setting pesticide tolerances. Under this new standard, EPA is to focus explicitly on pesticide exposures and risks for infants and children. The statute requires EPA to aggregate all sources of pesticide exposure when performing risk assessments and setting tolerances. This includes aggregating dietary exposure, drinking water exposure, nonoccupational exposure, and exposure from pesticides that share a common mechanism of toxicity. FQPA also mandated that EPA conduct a thorough reassessment of all existing tolerances within ten years. Organophosphate and carbamate insecticides have been placed in the highest priority tier for reassessment. Tolerances and registrations have already been cancelled under this reassessment process. Ultimately, the

Table 2. Insecticide end-use products registered by EPA for use on corn for control of corn rootworm species (CPR, 2000).

Product	Active Ingredients	Type [a]	Use Rate [b]	Use	Classification [c]
Ambush® *Insecticide* - Syngenta	permethrin – 25.6%	SP	0.2 lb/ac	Adult control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Asana® XL Insecticide 0.66 Emulsifiable Concentrate - DuPont	esfenvalerate – 8.4%	SP	0.05 lb/ac	Adult control	**WARNING. Restricted Use**: extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Aztec® 2.1% Granular Insecticide – Bayer Corp.	tebupirimfos – 2.0% cyfluthrin – 0.1%	OP SP	0.15 lb/ac 0.01 lb/ac	Larval control	**WARNING. Restricted Use**; toxic to fish and wildlife
Baythroid® 2 Emulsifiable Pyrethroid Insecticide - Bayer	cyfluthrin – 25%	SP	0.04 lb/ac	Adult control	**DANGER. Restricted Use**: extremely toxic to fish and aquatic invertebrates, highly toxic to bees, may cause allergic skin reactions
Capture® 2EC Insecticide/Miticide – FMC Corp.	bifenthrin – 25.1%	SP	0.3 lb/ac	Larval control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Chlorfos® 15G Insecticide Granular – Griffin LLC	chlorpyrifos – 15%	OP	2.02 lb/ac	Larval control	**CAUTION.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Chlorfos® 4E Insecticide – Griffin LLC	chlorpyrifos – 42%	OP	2.52 lb/ac	Adult & Larval control	**WARNING.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Counter® CR Systemic Insecticide-Nematicide – American Cyanamid Company	terbufos – 20%	OP	1.30 lb/ac	Larval control	**DANGER. Restricted Use**; fatal if swallowed, inhaled or absorbed through skin, extremely toxic to fish and wildlife
D-z-n® diazinon AG500 Insecticide - Syngenta	diazinon – 48%	OP	0.48 lb/ac	Adult control	**CAUTION. Restricted Use**: highly toxic to birds, fish and other wildlife, highly toxic to bees
D-z-n® diazinon AG600 WBC Insecticide - Syngenta	diazinon – 56%	OP	0.45 lb/ac	Adult control	**CAUTION. Restricted Use**: highly toxic to birds, fish and other wildlife, highly toxic to bees

Table 2 (cont.). Insecticide end-use products registered by EPA for use on corn for control of corn rootworm species (CPR, 2000).

Product	Active Ingredients	Type [a]	Use Rate [b]	Use	Classification [c]
Declare® *Emulsifiable Insecticide Concentrate* – Griffin LLC	methyl parathion – 45.11%	OP	0.22 lb/ac	Adult control	**DANGER. Restricted Use:** fatal if swallowed, inhaled or absorbed through skin, highly toxic to aquatic invertebrates and wildlife, highly toxic to bees
Diazinon 500-AG Organophosphate Insecticide – UAP	diazinon – 48%	OP	0.48 lb/ac	Adult control	**CAUTION. Restricted Use:** highly toxic to birds, fish and other wildlife, highly toxic to bees
Dimethoate 4 EC Systemic Insecticide - Helena	dimethoate – 44.8%	OP	0.45 lb/ac	Adult control	**WARNING.** Toxic to wildlife and aquatic invertebrates, highly toxic to bees
Dimethoate 400 Systemic Insecticide-Miticide - UAP	dimethoate – 43.5%	OP	0.44 lb/ac	Adult control	**WARNING.** Toxic to wildlife and aquatic invertebrates, highly toxic to bees
5 lb. Dimethoate Systemic Insecticide - Helena	dimethoate – 57%	OP	0.46 lb/ac	Adult control	**DANGER.** Toxic to wildlife and aquatic invertebrates, highly toxic to bees
Force® *3G Insecticide* - Syngenta	tefluthrin – 3%	SP	0.17 lb/ac	Larval control	**CAUTION. Restricted Use;** very highly toxic to freshwater and estuarine fish and invertebrates
Fortress® *2.5G granular insecticide* - DuPont	chlorethoxyfos – 2.5%	OP	0.16 lb/ac	Larval control	**DANGER. Restricted Use;** toxic to wild mammals, birds, fish and aquatic invertebrates
Fortress® *5G granular insecticide* – DuPont	chlorethoxyfos – 5%	OP	0.16 lb/ac	Larval control	**DANGER. Restricted Use:** toxic to wild mammals, birds, fish and aquatic invertebrates
Furadan® *4F insecticide/nematicide* – FMC Corp.	carbofuran – 44%	C	0.88 lb/ac	Adult & larval control	**DANGER. Restricted Use:** poisonous if swallowed or inhaled, toxic to fish, birds and other wildlife, highly toxic to bees, can seep or leach through soil and can contaminate groundwater

Table 2 (cont.). Insecticide end-use products registered by EPA for use on corn for control of corn rootworm species (CPR, 2000).

Product	Active Ingredients	Type [a]	Use Rate [b]	Use	Classification [c]
Lannate® LV insecticide – DuPont	methomyl - 29%	C	0.65 lb/ac	Adult control	**DANGER. Restricted Use**; fatal if swallowed, toxic to fish, aquatic invertebrates and mammals, highly toxic to bees, known to leach through soil into groundwater
Lannate® SP insecticide - DuPont	methomyl – 90%	C	0.45 lb/ac	Adult control	**DANGER. Restricted Use**: fatal if swallowed, may cause blindness, toxic to fish, aquatic invertebrates and mammals, highly toxic to bees, known to leach through soil into groundwater
Lorsban® 15G Granular Insecticide – Dow Agrosciences	chlorpyrifos – 15%	OP	2.03 lb/ac	Larval control	**CAUTION.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Lorsban® -4E Insecticide – Dow Agrosciences	chlorpyrifos – 44.9%	OP	2.69 lb/ac	Adult & larval control	**WARNING.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Mocap® 10% Granular Nematicide Insecticide – Aventis CropScience	ethoprop - 10%	OP	3.53 lb/ac	Larval control	**WARNING.** Toxic to aquatic organisms and wildlife
Mocap® EC Nematicide-Insecticide – Aventis CropScience	ethoprop – 69.6%	OP	3.34 lb/ac	Larval control	**DANGER. Restricted Use**: toxic to aquatic organisms and extremely toxic to birds
Penncap-M® Microencapsulated Insecticide – Elf Atochem	methyl parathion – 22%	OP	0.44 lb/ac	Adult control	**WARNING. Restricted Use**; highly toxic to aquatic invertebrates and wildlife
Phorate 20 G Organophosphate Insecticide - UAP	phorate – 20%	OP	1.3 lb/ac	Adult & larval control	**DANGER. Restricted Use**: extremely toxic to fish and wildlife
Pounce® WSB Insecticide – FMC Corporation	permethrin – 24.7%	SP	0.2 lb/ac	Adult control	**WARNING. Restricted Use**: extremely toxic to fish and aquatic invertebrates, highly toxic to bees

Table 2 (cont.). Insecticide end-use products registered by EPA for use on corn for control of corn rootworm species (CPR, 2000).

Product	Active Ingredients	Type [a]	Use Rate [b]	Use	Classification [c]
Pounce® 3.2 EC Insecticide – FMC Corporation	permethrin – 38.4%	SP	0.2 lb/ac	Adult control	**CAUTION. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Pounce® 25 WP Insecticide – FMC Corporation	permethrin – 25%	SP	0.2 lb/ac	Adult control	**WARNING. Restricted Use**: extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Regent® 4 SC Insecticide – Aventis CropScience	fipronil – 39.4%	PP	0.13 lb/ac	Larval control	**WARNING. Restricted Use**; toxic to birds, fish and aquatic invertebrates
Sevin® brand 80S Carbaryl Insecticide – Aventis CropScience	carbaryl – 80%	C	2.0 lb/ac	Adult control	**WARNING.** Extremely toxic to aquatic and estuarine invertebrates, highly toxic to bees
Sevin® brand 80WSP Carbaryl Insecticide – Aventis CropScience	carbaryl – 80%	C	2.0 lb/ac	Adult control	**WARNING.** Extremely toxic to aquatic and estuarine invertebrates, highly toxic to bees
Sevin® brand XLR PLUS Carbaryl Insecticide – Aventis CropScience	carbaryl – 44.1%	C	1.76 lb/ac	Adult control	**CAUTION.** Extremely toxic to aquatic and estuarine invertebrates, highly toxic to bees
Thimet® 20-G Soil and Systemic Insecticide – American Cyanamid	phorate – 20%	OP	1.3 lb/ac	Larval control	**DANGER. Restricted Use**; extremely toxic to fish and wildlife
Warrior® Insecticide with Zeon Technology - Syngenta	lambda-cyhalothrin – 11.4%	SP	0.03 lb/ac	Adult control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic organisms and toxic to wildlife, highly toxic to bees

a - OP organophosphate, SP synthetic pyrethroid; C carbamate, PP phenyl pyrazole
b – maximum labeled use rate expressed in pounds of active ingredient per acre (assume that 1 liq pt ~ 1 lb)
c – precautionary language as stated on label

FQPA reassessment process may result in the loss of some active ingredients for corn rootworm control.

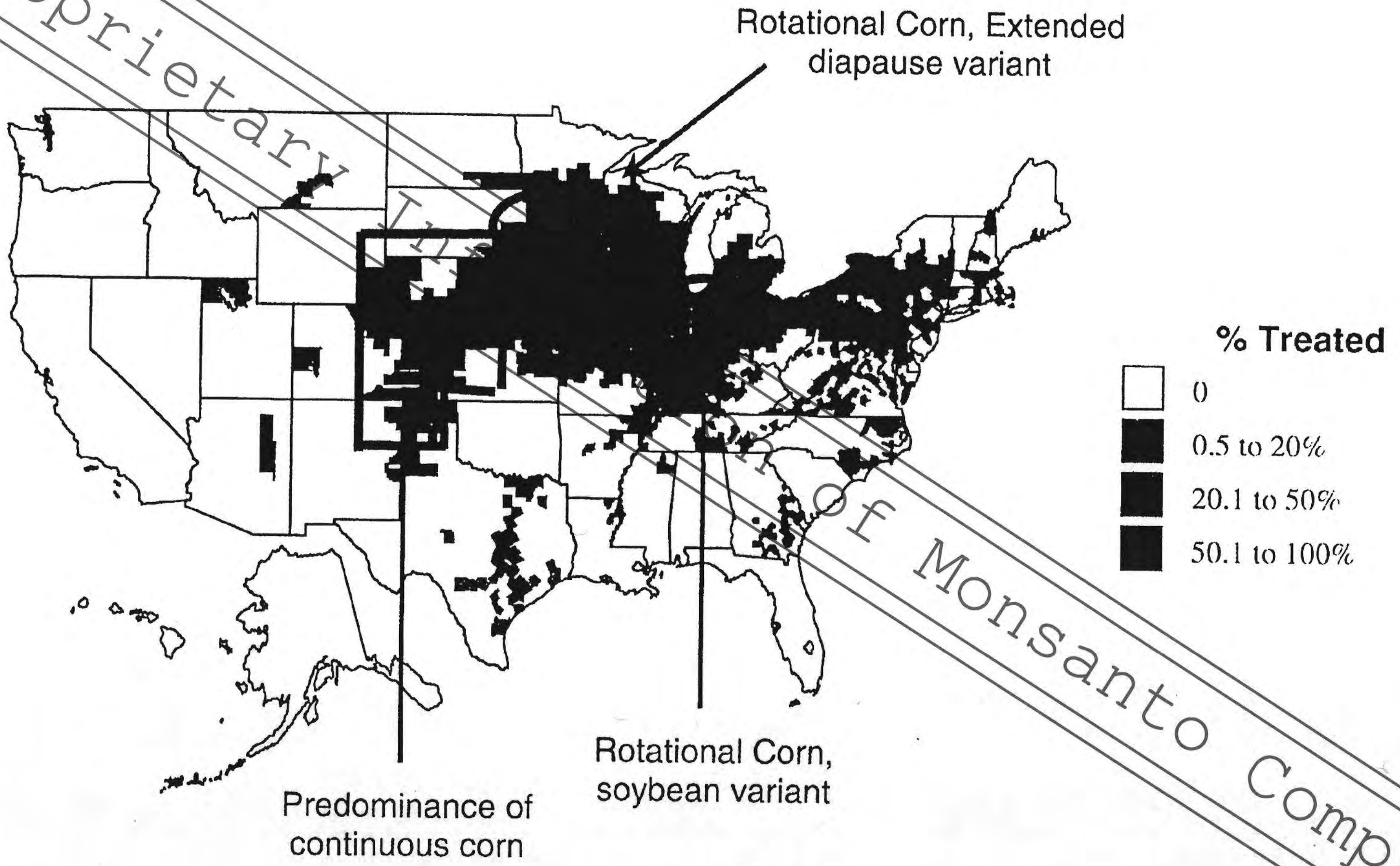
Doane Marketing Research, Inc., has conducted a comprehensive study of pesticide use on select row, field and specialty crops grown in the continental U.S. for year 2000. In this study, The 2000 AgroTrak Study, data was collected and reported on a crop-specific basis. Data were collected primarily by telephone interviews of growers. Mail was used on a supplemental basis. All data were weighted for each crop at the state and crop reporting district level to project national totals. The weighting process used a multiple regression procedure to derive best-fit estimates by adjusting for known disproportionalities of the respondents. Individual crop acreage estimates were weighted to the latest USDA and state estimates. This data is searchable in the syndicated AgroTrak database. This database has been used to estimate the type and quantity of insecticides applied for control of corn rootworm. The Doane's database is being used for this analysis in lieu of the NASS database because it is more comprehensive in scope and can be easily searched on-line.

In year 2000, growers reported that 14,197,012 acres of corn were treated with an insecticide for the control of corn rootworm. Growers indicated that corn rootworm was the sole target pest on approximately half of these acres (6,957,678) and one of a number of pests targeted for control on the remaining acres. Corn rootworm-targeted acres that received an insecticide treatment represented 17% of the total acres of corn planted in the continental U.S. and 59% of the total acres receiving any insecticide treatment in 2000. Continuous corn and first year corn acres received 58% and 42% of the rootworm-targeted insecticide applications, respectively. A total of 7,835,995 pounds of insecticide active ingredient (a.i.), costing $172 million, was applied to these acres. Table 3 contains a summary of year 2000 corn insecticide use data. Figure 11 displays the regional distribution of corn rootworm insecticide-treated acres in the continental U.S. This map clearly shows that the percentage of planted acres receiving treatment is highest in continuous corn regions and in the region where the western corn rootworm soybean variant is prevalent.

Table 3. Insecticide usage on corn in year 2000 (Doane, 2001).

Parameter	Continuous Corn	First Year Corn	All Corn
Acres planted (x1,000)	22,269	57,310	79,579
Total insecticide treated acres (x1,000)	11,590	12,518	24,108
Total insecticide a.i. applied (lb x1,000)	6,332	6,011	12,343
CRW-targeted acres (x1,000)	8,271	5,926	14,197
a.i. applied to CRW-targeted acres (lb x1,000)	4,699	3,137	7,836
Average a.i. rate applied (lb/ac)	0.568	0.529	0.552
Average cost per acre	$11.95	$12.27	$12.08
Total cost of CRW insecticide purchased (x1,000)	$98,811	$72,699	$171,510

Figure 11. Corn acres treated with an insecticide for control of corn rootworm expressed as a percent of total planted acres in year 2000 (Doane, 2001).



Many products are registered for the control of corn rootworm (see Table 2) but a small number dominate the market. Table 4 provides a listing of the active ingredients contained in products used for treatment of corn rootworm-targeted acres. Four active ingredients, chlorpyrifos, tebupirimfos, tefluthrin and terbufos, are applied to 75% of the acres treated. Three of these active ingredients are organophosphates and three are classified as restricted use. Two active ingredients, chlorpyrifos and terbufos, account for 77% of the total quantity of insecticide applied to corn rootworm-targeted acres. Table 5 provides a listing of the major end-use products that are applied to corn rootworm-targeted acres. Applications with the 13 products listed in Table 5 account for 98% of the corn rootworm-targeted acres that were treated in 2000. The use of five products, *Aztec 2.1% Granular Insecticide*, *Counter CR Systemic Insecticide-Nematicide*, *Force 3G Insecticide*, *Lorsban 15G Granular Insecticide*, and *Regent 4 SC Insecticide*, accounted for applications to 83% of the rootworm-targeted acres. Four of these five products are restricted use pesticides. Control of corn rootworm larvae, as opposed to control of adults, was by far the prevalent pest control strategy utilized by growers.

Table 4. Insecticide active ingredients applied to corn rootworm-targeted acres in year 2000 (Doane, 2001). These 11 active ingredients accounted for 98.3% of the total quantity of insecticide applied to corn rootworm-targeted acres in 2000.

Active Ingredient	Acres Treated (x1,000)	Pounds Applied
Carbofuran	342	242,379
Chlorethoxyfos	361	55,485
Chlorpyrifos	3,557	3,765,310
Cyfluthrin/Tebupirimfos	1,326	179,527
Fipronil	1,498	158,141
λ-cyhalothrin	179	3,846
Methyl parathion	367	142,011
Permethrin	246	24,344
Phorate	508	588,380
Tefluthrin	3,570	400,339
Terbufos	2,044	2,146,761
Total	13,998	7,706,523

A small number of seed-applied insecticides have recently entered the corn insect control market. These products include *Gaucho® seed-applied insecticide* (Gustafson, LLC), *Prescribe™ seed-applied insecticide* (Gustafson, LLC) and *Force® ST seed-applied insecticide* (Syngenta). Imidacloprid is the active ingredient in both *Gaucho* and *Prescribe*; tefluthrin is the active ingredient in *Force ST*. *Gaucho* is applied to corn seed at a rate of 0.16 mg a.i./kernel and provides protection from damage and stand loss caused by wireworms, seedcorn maggots, white grubs and imported fire ants (*i.e.*, secondary soil pests). This seed-applied rate of 0.16 mg/kernel equates to 0.011 pounds of a.i./ac planted. By contrast, the average soil-applied insecticide rate of 0.552 lb/ac is 50x higher than the seed-applied rate. *Prescribe* is applied to seed at a higher rate of 1.34 mg a.i./kernel and *Force® ST seed-applied insecticide* is applied at a rate of 1.0 mg a.i./kernel. Both *Prescribe* and

Table 5. Insecticide end-use products used for control of corn rootworm in year 2000 (Doane, 2001).

Product [a]	Average Cost ($/ac)	Adult (A) / Larval (L) Control	EPA Classification	Acres Treated (x1,000)
Aztec 2.1% Granular Insecticide (tebupirimfos/cyfluthrin)	$13.05	L	Restricted	1,327
Counter CR Systemic Insecticide-Nematicide (terbufos)	$13.10-$13.50	L	Restricted	2,044
Force 3G Insecticide (tefluthrin)	$14.48	L	Restricted	3,570
Fortress 5G granular insecticide (chlorethoxyfor)	$14.65	L	Restricted	361
Furadan 4F insecticide/ nematicidel (carbofuran)	$11.74	L	Restricted	342
Lorsban 15G Granular Insecticide (chlorpyrifos)	$11.79	L	Unrestricted	3,165
Lorsban 4E Insecticide (chlorpyrifos)	$10.52	A	Unrestricted	374
Penncap-M Microencapsulated Insecticide (methyl parathiod	$6.79	A	Restricted	330
Pounce 3.2 EC Insecticide (permethrin)	$4.42	A	Restricted	224
Regent 4 SC Insecticide (fipronil)	$14.65	L	Restricted	1,392
Regent 80 WG Insecticide (fipronil)	$8.57	L	Restricted	106
Thimet 20-G Soil and Systemic Insecticide (phorate)	$10.90-$12.74	L	Restricted	508
Warrior Insecticide with Zeon Technology (λ-cyhalothrin)	$7.37	A	Restricted	173
Total				13,916
Adult Control	$4.42 – $10.52			8%
Larval Control	$8.57 - $14.65			92%
Restricted Use				73%
Unrestricted				27%

a – active ingredient(s) stated in parentheses

Force ST claim protection from damage caused by corn rootworms as well as other soil insects of secondary economic importance. The results of Monsanto efficacy trials, as well as independent trials of Steffey and Gray (2000), indicate that *Prescribe* and *Force ST* do not control corn rootworm damage as well as most soil-applied insecticides. Performance is inconsistent and weak under conditions of high corn rootworm pressure. There is also an issue with shelf-life of seed treated with *Prescribe*; most of this seed does not remain viable if carried over into a second planting season (Monsanto storage stability data).

V. *B.t.* Protein Cry3Bb1 and Corn Event MON 863

In 1991, Rupar *et al.*, reported discovery of a novel *Bacillus thuringiensis (B.t.)* strain (EG4691) that produced a crystal protein that displayed activity against the southern corn rootworm. Donovan *et al.* (1992), isolated and sequenced the gene encoding this crystal protein, which was designated Cry3Bb1 (GenBank Accession No. M89794). This wild type Cry3Bb1 protein is present in the commercial product, *Raven*[*] *Oil Flowable Bioinsecticide*, which has been sold in the U.S. since 1995 for control of the Colorado potato beetle, a coleopteran pest. *Raven* has never been commercialized for corntrol of corn rootworm or any other pest of corn. Cry3Bb1 shares approximately 67% amino acid sequence identity with another Cry3 protein, Cry3Aa4 (GenBank Accession No. M30503), which has been commercially used in the U.S. and other countries for control of the Colorado potato beetle, a major pest of potatoes (Perlak *et al.*, 1993).

Advanced molecular techniques have been directed to the design of genes that encode proteins with enhanced insecticidal activity. English *et al.* (2000), have designed multiple genes encoding Cry3Bb1 variants with enhanced activity against corn rootworm species. These variants are virtually identical in structure to the Cry3Bb1 wild type protein with the exception of a small number of strategically placed amino acid substitutions that enhance insecticidal activity. These genes have been linked to constitutive plant expressible promoters and codon-optimized for expression in monocotyledonous plants in order to achieve meaningful levels of protein production in root tissues (Fischoff and Perlak, 1996; Brown *et al.*, 1997).

Corn event MON 863 was created by particle acceleration technology using a linear DNA fragment containing synthetic *cry3Bb1* coding sequence, as well as coding sequence for the selectable marker, neomycin phosphotransferase type II (NPTII). This linear DNA fragment has been designated as vector ZMIR13L. Incorporation of this vector into the corn genome results in the production of a Cry3Bb1 variant that shares 98.9% sequence identity with the wild type crystal protein. Monsanto Company has conducted extensive research to characterize corn event MON 863 and to define potential human and environmental hazards associated with Cry3Bb1 protein.

A. Product Characterization

Southern blot analyses have demonstrated that corn event MON 863 contains one copy of the transformation vector inserted at a single locus in the plant genome (Cavato *et al.*, 2001). No additional elements from the ZMIR13L vector, linked or unlinked to intact gene cassettes, were detected in the plant genome. These findings confirm that corn containing event MON 863 will produce the full length proteins Cry3Bb1 and NPTII. Segregation analysis of the corn rootworm-protected phenotype and Southern blot fingerprint analysis across multiple generations have confirmed the stability of the inserted genes in MON 863 corn (Ward, 2001).

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[*] A trademark of Ecogen, Inc.; EPA Reg. No. 55638-27

Nucleotides comprising the transgenic insert in event MON 863 have been sequenced by Monsanto (Hileman and Astwood, 2001a). The amino acid sequence of the Cry3Bb1 variant produced in corn event MON 863 has been deduced from the nucleotide sequence and confirmed by N-terminal sequencing and matrix assisted laser desorption ionization time of flight mass spectroscopy (Thoma *et al.*, 2001). The Cry3Bb1 variant produced in MON 863 corn differs from the wild type Cry3Bb1 protein by seven amino acids (Hileman and Astwood, 2001a). Structural data (*i.e.*, x-ray crystallography resolved to 2.4 A) have shown that this small number of amino acid substations does not alter overall three-dimensional structure of the protein (Astwood *et al.*, 2001).

Levels of Cry3Bb1 produced in various tissues of MON 863 corn have been found to be in the range of 3 to 93 μg/g of fresh tissue weight (Dudin *et al.*, 2001). Cry3Bb1 levels in root tissue are sufficient to confer protection against significant corn rootworm feeding damage. Agronomic observations recorded in multiple field trials conducted across major corn growing regions of the U.S. have demonstrated that corn containing event MON 863 is phenotypically equivalent to conventional corn except for its tolerance to corn rootworm larval feeding (Pilcher *et al.*, 2001).

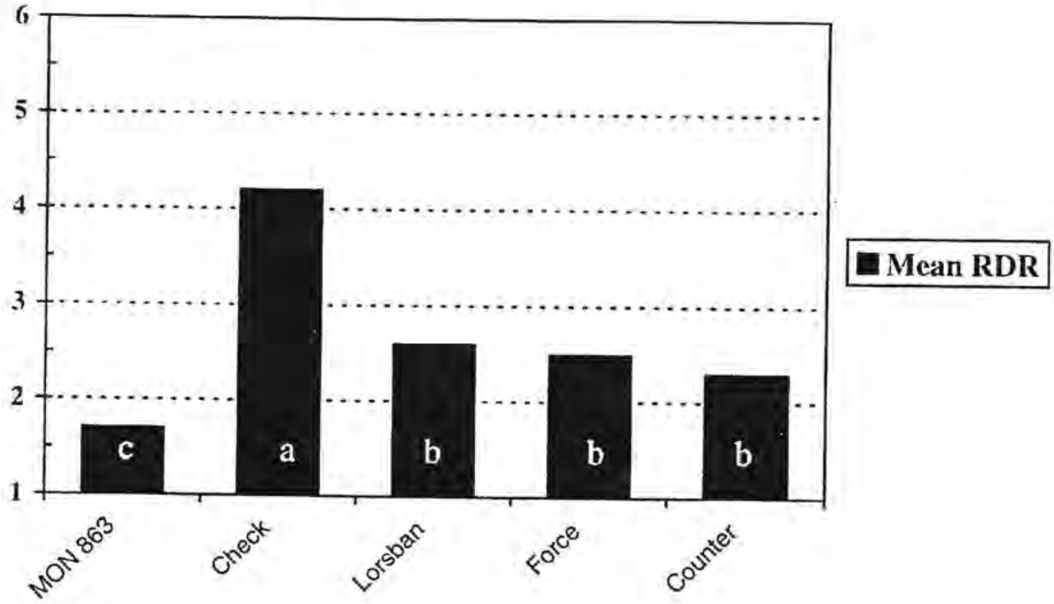
B. Product Efficacy

The two most economically important *Diabrotica* pests in corn crops are the western corn rootworm and the northern corn rootworm. Three years of Monsanto and university field research have conclusively demonstrated the efficacy of event MON 863 in limiting the extent of root damage caused by corn rootworm larval feeding. The results of 1999 and 2000 field efficacy trials have previously been submitted to EPA (Ward, 2000; Pilcher, 2001).

In replicated randomized block efficacy trials conducted at multiple locations by university researchers, the level of root damage caused by western and northern corn rootworm larval feeding was evaluated in hybrids containing event MON 863, in comparable nontransgenic and untreated control hybrids, and in hybrids treated with leading soil-applied insecticide brands. One seed-applied insecticide was evaluated in 2001 trials. Mean root damage ratings (RDR) were computed for each treatment. Statistically significant differences between mean ratings were identified by an analysis of variance procedure ($p<0.05$). A summary of 2000 and 2001 efficacy trial results can be found in Figures 12 and 13, respectively.

The results of these efficacy field trials demonstrate that corn hybrids containing event MON 863 consistently limits larval feeding damage to levels below economic thresholds. The estimated root rating at which yield caused by larval feeding is equal to or greater than the cost of insecticidal control can range from 2.5 to 4.0 (Steffey *et al.*, 1999). MON 863 varieties consistently outperformed commercial insecticide standards. Soil insecticides require rain or irrigation to be activated and their performance can be greatly impacted by timing of application. If weather conditions permit, early planting of corn generally leads to increased yields. However, if soil insecticides are applied too far in advance of rootworm hatch (generally in late May) insufficient concentrations may remain to effectively control larval feeding damage. Excessive soil moisture or rainfall can also diminish insecticide performance. Neither of these limitations applies to varieties containing event MON 863. Since the Cry3Bb1 insecticide is contained in the root tissue, it does not require activation and levels are highest at the period when larvae are actively feeding (Dudin *et al.*, 2001).

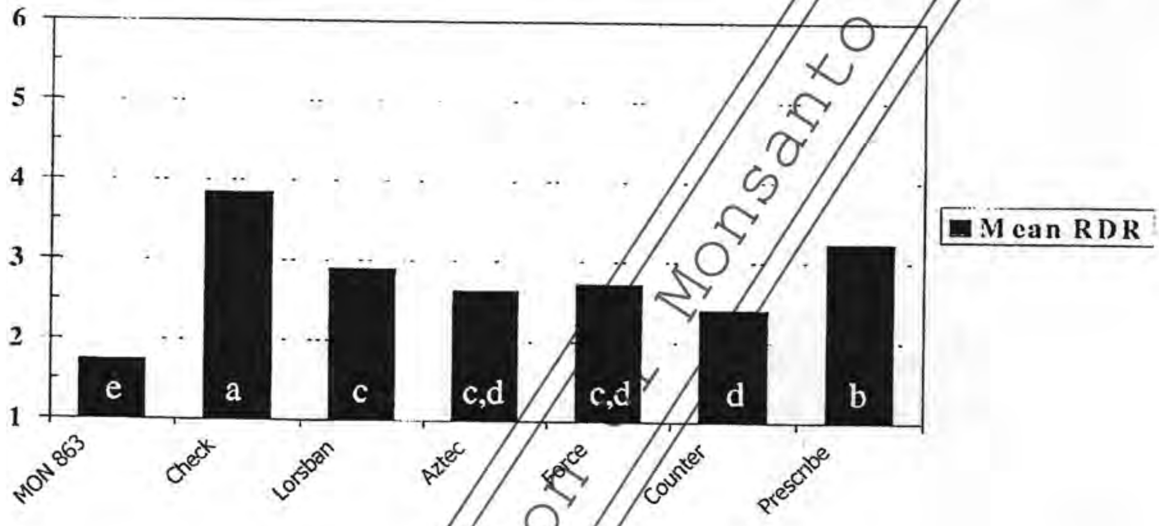
Figure 12. Summary results of year 2000 efficacy trial results with corn hybrids containing event MON 863. Individual roots were dug and rated based on the Iowa 1 to 6 RDR scale. The mean RDR for trials conducted at 14 locations were computed (N=280 roots/treatment). Significant differences at $p<0.05$ are denoted by letter code: 'b' is significantly different than 'a'; 'c' is significantly different than 'a' and 'b'.



In 2001, *Prescribe seed-applied insecticide* was included in the efficacy trials conducted by university researchers (see Figure 13). This seed treatment provided some level of control compared to untreated checks, but its performance was notably weaker than each of the soil-applied products evaluated and weaker than MON 863. In a separate set of efficacy trials (data not shown), seed treated with the lower rate of imidacloprid contained in *Gaucho seed-applied insecticide* provided excellent control of secondary soil insects (*e.g.* wireworms, white grubs and seed corn maggots).

The distribution of individual plant root ratings can provide a measure of insecticide performance consistency. Although a mean RDR may be below the economic threshold for an insecticide treatment, there can be considerable variability in root feeding damage within a given field. Pruning of one or more roots, which is equivalent to a RDR of 3, causes sufficient damage to result in a yield loss for an individual plant. Any plant that has root pruning at a level equivalent to a RDR ≥4 is at risk of lodging. Plant lodging greatly increases the time and complexity of harvesting a field. Figure 14 displays the distribution of individual root ratings recorded in 2000 and 2001 efficacy trials with MON 863 hybrids and

Figure 13. Summary results of year 2001 efficacy trial results with corn hybrids containing event MON 863. Individual roots were dug and rated based on the Iowa 1 to 6 RDR scale. The mean RDR for trials conducted at 21 locations were computed (N=465 roots/treatment). Significant differences at $p<0.05$ are denoted by letter code: 'b' is significantly different than 'a'; 'c' is different than 'a' and 'b'; 'd' is different than 'a', 'b' and 'c'; 'e' is different than 'a', 'b', 'c' and 'd'.



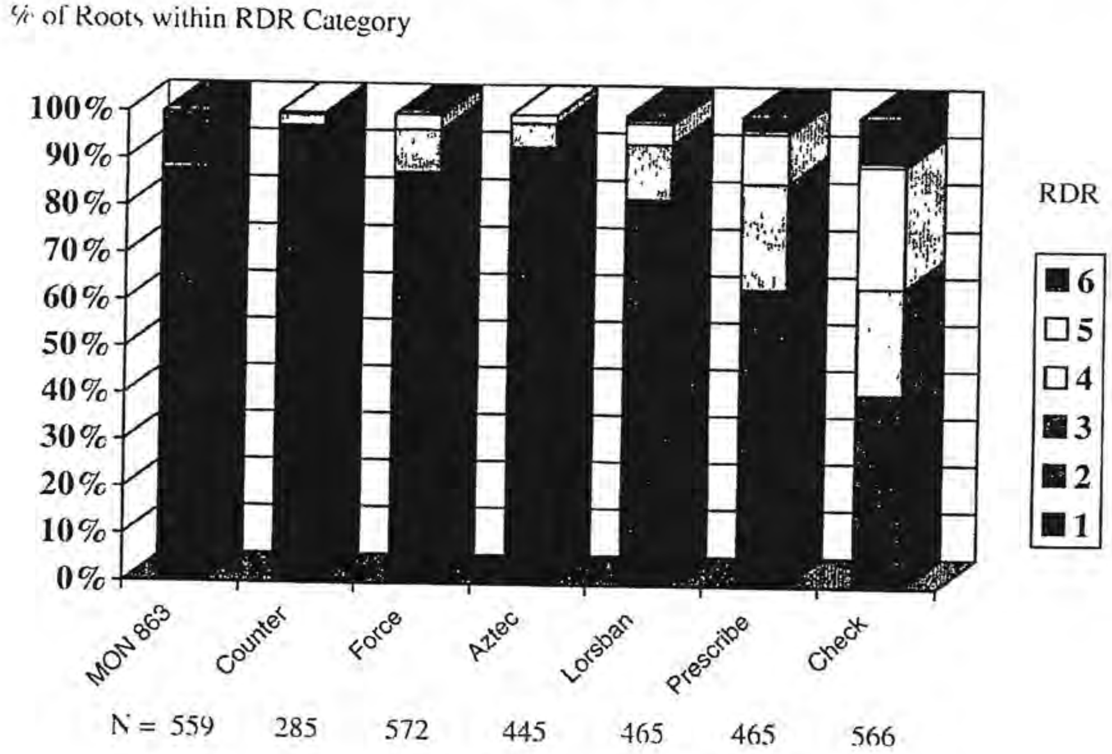
insecticide standards. This analysis demonstrates that MON 863 consistency of performance is superior to that of the soil and seed-applied insecticides.

Data from field trials in which the average check RDR was greater than three were used in this analysis of performance consistency. An average RDR>3 is indicative of significant rootworm pressure in a field. It is not possible to identify differences in product performance at larval infestation levels below the economic threshold. A total of 559 MON 863 roots were rated and included in this analysis. Only 12% of the MON 863 plants rated had a RDR=3; not a single plant was found with root damage exceeding a RDR of 3. In contrast, of the 465 plants treated with *Lorsban 15G*, a widely used insecticide for corn rootworm control, 80% of plants had a RDR≥3 and a small percentage of plants received the maximum RDR of 6. Consistency of performance was weakest with the seed treatment, *Prescribe*.

Large plot trials planted under diverse environmental conditions are needed to demonstrate definitive yield differences for corn. It has not been practical to conduct such trials under USDA performance standards or the restrictions of an experimental use permit (524-EUP-93). However, it is fully expected that the superior protection of roots provided by Cry3Bb1 compared to soil-applied insecticides will translate into a meaningful yield advantage for growers when this product is commercialized.

In a multiyear study to investigate the impact of corn rootworm larval feeding on compensatory root growth and yield of nontransgenic corn, Gray and Steffey (1998a) were

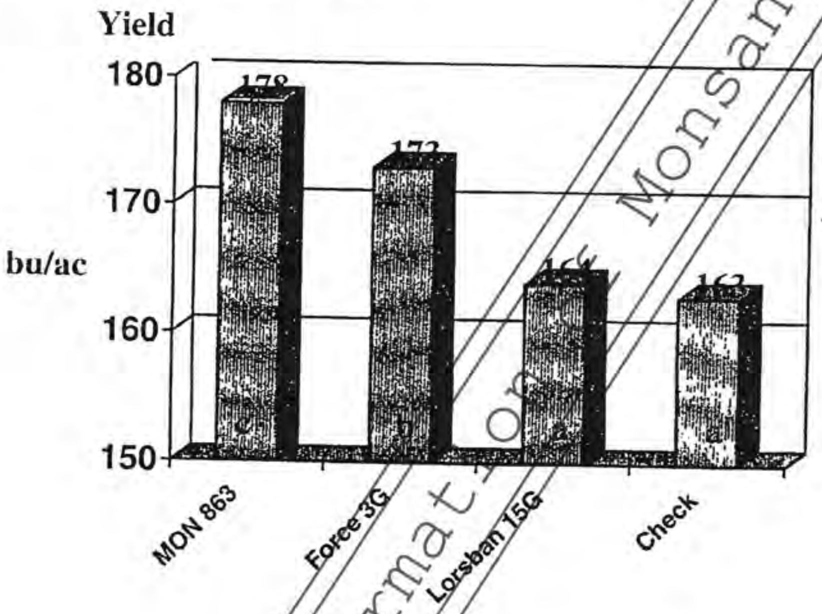
Figure 14. Distribution of individual plant RDR scores evaluated in 2000 and 2001 university efficacy trials. Root feeding damage was scored using the Iowa root damage rating (RDR) 1 to 6 scale. Data are presented for trials in which the average check RDR >3.0. Without significant corn rootworm pressure in a field it isn't possible to distinguish between treatments. (N) is the number of individual plants rated for each treatment. *Aztec 2.1G* and *Prescribe* data are for 2001 only.



able to demonstrate through a regression analysis that incremental reductions in RDR scores equated to a yield benefit for growers. Mitchell (2002) has used the Gray and Steffey field data to estimate proportional yield loss as a function of the root rating difference. Applying this model to the data from efficacy field trials with hybrids containing event MON 863 provides an estimate of the yield benefit for event MON 863 relative to no corn rootworm control and relative to control with a soil-applied insecticide. Over typical ranges for corn rootworm pressure, MON 863 hybrids provide a yield benefit of 9 to 28% relative to no pest control and 1.5 to 4.5% relative to control with leading soil insecticides. For a reasonable range of prices and yields, the value of the event MON 863 yield benefit is $25-$75/ac relative to no pest control and $4-$12/ac relative to control provided by a soil insecticide.

Preliminary data obtained from small plot trials conducted by Monsanto at multiple locations in 2001 corroborate the Mitchell prediction of a yield advantage for MON 863 hybrids. These small plot trials demonstrated a yield benefit for MON 863 over two leading soil-applied insecticides that ranged from five to 14 bu/ac (refer to Figure 15). Although the yield differences noted here are small, they do appear to be attributable to superior root protection afforded by the MON 863 hybrids. Lodging was not observed in these small plot trials.

Figure 15. Summary results from small plot yield comparison trials with four MON 863 hybrids and two leading soil-applied insecticides. MON 863 and *Force 3G* trials were conducted at 19 locations. *Lorsban 15G* was evaluated at 13 locations. The average RDR in check plots was 3.2. Significant yield differences at $p<0.05$ are denoted by letter code: 'b' is significantly different than 'a'; 'c' is different than 'a' and 'b'.



C. Mammalian Safety

B.t. is a gram-positive bacterium commonly present in soil that has been used commercially in the U.S. since 1958 to produce microbial-derived products with insecticidal activity (EPA, 1988). The extremely low mammalian toxicity of *B.t.*-based insecticide products has been demonstrated in numerous safety studies (McClintock *et al.*, 1995). The results of studies filed in support of the application to register Cry3Bb1, as well as the results of studies conducted with other Cry3 proteins, demonstrate that this class of proteins is essentially nontoxic to mammals.

Data requirements for *B.t.* proteins produced in genetically modified crops include acute oral toxicity and *in vitro* digestibility studies. These requirements are based on the fact that oral ingestion is the only meaningful route of exposure for humans to Cry proteins in genetically improved crops. Furthermore, when proteins are toxic they are known to act *via* acute mechanisms and at very low dose levels (Sjoblad *et al.*, 1992). The results of rodent acute oral toxicity tests conducted with Cry3 proteins, including several Cry3Bb1 variants, are summarized in Table 6. In each rodent bioassay, the highest achievable dose level failed to produce evidence of treatment related adverse effects and was, therefore, considered to be a no observable effect level (NOEL).

Table 6. Rodent acute oral NOELs determined for various Cry3 proteins. The highest dose tested in each bioassay was found to be a NOEL.

Cry3 Protein	NOEL (mg/kg)	Reference
Cry3Bb1 - MON 863 variant	≥ 3200	Bonnette and Pyla, 2001
Cry3Bb1 - EG11098 variant	> 2980	Bectel, 1999a
Cry3Bb1 - EG11231 variant	≥ 3780	Bectel, 1999b
Cry3Bb1 – Wild type present in *Raven Oil Flowable Bioinsecticide* [1]	≥ 30	Baum *et al.*, 1996
Cry3Aa4 - *NewLeaf*® *Potato*	≥ 5000	Lavrik *et al.*, 1995

1 *Raven Oil Flowable Bioinsecticide* contains a mixture of Cry3Bb1, Cry3Aa4 and Cry1Ac proteins. In the batch tested, Cry3 proteins constituted 40% (wt/wt) of the formulation; Cry3Bb1 protein comprised 66-75% of the Cry3 proteins present in *Raven*. The highest dose tested was 10^8 CFU/rat, which corresponds to approximately a 100 mg/kg body weight dose of total active ingredients.

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The identical *cry3Bb1* coding sequence used in construction of vector ZMIR13L was inserted into a strain of *E. coli* that was used in a heterologous fermentation system to produce the test material for MON 863 toxicity studies. As expected, the Cry3Bb1 protein purified from this fermentation system was found to be physicochemically and functionally equivalent to the protein produced in MON 863 corn (Hileman *et al.*, 2001). In the acute oral toxicity study performed with laboratory mice, no mortality or grossly observable adverse effects were noted (Bonnette and Pyla, 2001). The NOEL was determined to be ≥3,200 mg/kg, which was the highest dose tested.

Monsanto has previously submitted to EPA the results of acute rodent toxicity studies with two other variants of Cry3Bb1. These variants were genetically engineered to enhance insecticidal activity against corn rootworm and were produced by the recombinant *B.t.* strains, EG11098 and EG11231. They have been found to be functionally and physicochemically equivalent to the variant of Cry3Bb1 produced in MON 863 corn (Astwood *et al.*, 2001). The EG11098 and EG11231 variants of Cry3Bb1 differ from the MON 863 variant by two and three amino acids, respectively. Sequence homology for the three variants is >99.5%.

Current scientific knowledge suggests that food allergens tend to be resistant to proteolytic digestion, acid and heat, and may be glycosylated. The results of *in vitro* digestive fate studies indicate that Cry3Bb1 proteins degrade to nondetectable levels in simulated gastric fluid in less than one minute (Leach *et al.*, 2001a and 2001b). Neither the Cry3Bb1 protein produced by *E. coli* or MON 863 corn is glycosylated (Hileman *et al.*, 2001). Cry3Bb1 is not detectable in MON 863 grain following baking at typical grain processing temperatures (*i.e.* 204°K) for 30 minutes (Holleschak *et al.*, 2001). A comparison of amino acid sequences for known protein allergens and toxins revealed no biologically relevant structural similarities to the Cry3Bb1 protein produced in corn event MON 863 (Hileman and Astwood, 2001b).

High-dose acute exposure studies are considered appropriate for assessing the potential toxicity of Cry proteins to mammals. EPA scientists have stated: "if toxic proteins are known to act through acute mechanisms. Also, laboratory animals show acute toxic effects from exposure to proteins known to be toxic to humans" (Sjoblad *et al.*, 1992). The potential for human exposure to Cry3Bb1 will occur through consumption of corn or corn products containing the protein. Exposure *via* the dermal or inhalation route is unlikely since the protein is contained within the plant tissue. There appears to be little opportunity for occupational exposure to the active ingredient. The protein has been found to be short-lived in soil, thus runoff to drinking water sources will be negligible. A margin of exposure (MOE) for Cry3Bb1 protein has been computed based on estimates of corn dietary intake. The MOE is defined as the ratio of an appropriate NOEL to an estimate of human daily dietary exposure (DDE). An upper bound estimate of human daily corn consumption has been obtained from the Exposure-1 Chronic Dietary Exposure Analysis Program. The estimate of daily human corn consumption is 0.2 g corn per kg body weight and the highest level of Cry3Bb1 found in samples of corn grain is 0.086 mg protein / g of grain. The MOE is computed as follows:

Daily corn consumption (g/kg) x Cry3Bb1 grain concentration (mg/g) = DDE (mg/kg)
NOEL (mg/kg) ÷ DDE (mg/kg) = **MOE**

Computation of the MOE for Cry3Bb1 in MON 863 corn is as follows:

$$0.2 \text{ g/kg} \times 0.086 \text{ mg/g} = 0.017 \text{ mg/kg}$$
$$3{,}200 \text{ mg/kg} \div 0.017 \text{ mg/kg} = \mathbf{1.9 \times 10^5}$$

A MOE of ≥100 is generally regarded as being protective of human health. There is a five order of magnitude difference between the acute oral NOEL and the upper bound estimate of human dietary exposure to Cry3Bb1 in MON 863. This large margin of exposure ensures a reasonable certainty of no harm for humans and other mammals exposed to the product.

On May 11, 2001, an exemption from the requirement for a tolerance was established for Cry3Bb1 protein and the genetic material necessary for its production in corn (EPA, 2001a). As part of the decision to grant this tolerance exemption, EPA stated: "The lack of mammalian toxicity at high levels of exposure to the Cry3Bb1 [. . .] proteins demonstrate the safety of the product at levels well above maximum exposure levels anticipated in the crop." The Agency further stated: "There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry3Bb1 [. . .] proteins and the genetic material necessary for their production. This includes all anticipated dietary exposures and other exposures for which there is reliable information." On December 31, 2001, Monsanto completed the Premarket Consultation Process with the Food and Drug Administration (FDA) for corn event MON 863.

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Completion of this consultation process has resulted in a conclusion that corn derived from this new variety is not materially different in composition, safety, and other relevant parameters from corn currently on the market.

In summary, the human safety of Cry3Bb1 protein is demonstrated by: 1) the results of studies that show an absence of adverse effects in a mammalian specie following acute oral exposure, a very large margin of exposure for projected dietary consumption, rapid digestion, and the absence of sequence similarities to known toxins and allergens; 2) comparable results to studies of other proteins in the Cry3 class, including Cry3Bb1 in the commercial product, *Raven Oil Flowable Bioinsecticide*; and 3) a nearly 50-year history of safe use for *B.t.* Cry proteins in U.S. agriculture. The weight of evidence establishes a reasonable certainty of no harm for this protein.

Both EPA and the U.S. Food and Drug Administration have previously determined that NPTII is safe for human and animal consumption (EPA, 1994; FDA, 1998).

D. Environmental Safety

The insecticidal specificity of a Cry protein can be determined by any number of processes in its mechanism of action, including solubilization of the crystal, proteolytic processing, stability of the toxin, receptor affinity, and the formation of ion channels and pores within that membrane (English and Slatin, 1992). Receptor binding, in particular, is a critical step in the mechanism of action of the Cry proteins because without a binding site no toxic effect can be exerted. Irreversible binding of toxins to midgut receptors appears to be correlated with insect susceptibility (Schnepf *et al.*, 1998). This is an important factor in assessing the safety of Cry proteins for nontarget organisms such as fish, birds and mammals. To data, no functional receptors for these proteins have been identified on intestinal cells of fish, birds or mammals (Noteborn, 1994; Sacchi *et al.*, 1986; Van Mellaert *et al.*, 1988).

B.t. Cry proteins have been generally classified based on their insecticidal activity. For example, Cry1 proteins are toxic to lepidopteran pests, Cry2 proteins are toxic to lepidopteran and dipteran pests, Cry3 proteins are toxic to coleopteran pests, and Cry4 proteins are toxic to dipteran pests (Bravo, 1997; Höfte and Whiteley, 1989). The Cry3 class protein, Cry3Bb1, 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 for Cry3Bb1. These bioassays support the argument that Cry3Bb1 insecticidal activity is limited to beetle species within the family Chrysomelidae (Head *et al.*, 2001).

Potential adverse effects on nontarget organisms resulting from exposure to Cry3Bb1 have been evaluated in a series of studies with representative avian, aquatic and terrestrial beneficial invertebrate species. These nontarget organisms were exposed to high doses of leaf tissue, grain or pollen containing a plant-produced Cry3Bb1 variant or to an artificial diet containing a *B.t.*-produced Cry3Bb1 variant. The results of these laboratory bioassays are summarized in Table 7. In each case a no observable effect concentration (NOEC) for Cry3Bb1 was established.

Table 7. Summary of results from ecological effects tests with Cry3Bb1 proteins. For assays utilizing plant tissue as the carrier, either the MON 863 or MON 853 variant of Cry3Bb1 was the test material. All other bioassays employed an artificial diet containing the EG11231 variant of Cry3Bb1. Risk conclusions are based on protein concentrations measured in plant tissues of corn event MON 863 (Dudin *et al.*, 2001).

Test Organism	Test Substance	Results [a]	Conclusions [b]	Reference
Cladoceran (*Daphnia magna*)	Pollen	NOEC ≥ 2.26 µg/l	NOEC ≥ 141x surface water MEEC	Drottar and Krueger, 1999
Collembola (*Folsomia candida*)	Leaf	NOEC > 872.5 µg/g	NOEC > 66x soil MEEC	Teixeira, 1999
Channel Catfish (*Ictalurus punctatus*)	Grain	No effect on growth or survival at 35% of diet	No significant risk	Li and Robinson, 1999
Bobwhite Quail (*Colinus virginianus*)	Grain	No effect on growth or survival at 10% of diet	No significant risk	Gallagher *et al.*, 1999
Adult Honey Bee (*Apis mellifera*)	Purified protein	NOEC ≥ 360 µg/ml	NOEC ≥ 3.8x maximum pollen level	Maggi, 1999a
Larval Honey Bee (*Apis mellifera*)	Purified protein	NOEC ≥ 1790 µg/ml as a single dose	NOEC > 19x maximum pollen level	Maggi, 1999b
Adult Ladybird Beetle (*Hippodamia convergens*)	Purified protein	NOEC > 8000 µg/g	NOEC ≥ 86x maximum pollen level	Palmer and Krueger, 1999c
Adult Ladybird Beetle (*Hippodamia convergens*)	Pollen	No effect on growth or behavior at 50% of diet	No significant risk	Bryan *et al.*, 2001
Larval Ladybird Beetle (*Coleomegilla maculata*)	Pollen	No effect on growth or survival at 50% of diet	No significant risk	Duan *et al.*, 2001a
Adult Ladybird Beetle (*Coleomegilla maculata*)	Pollen	No effect on survival at 50% of diet	No significant risk	Duan *et al.*, 2001b
Monarch Butterfly Larvae (*Danus plexippus*)	Pollen	No effect on growth or survival	No significant risk	Sears and Mattila, 2001
Green Lacewing Larvae (*Chrysoperla carnea*)	Purified protein	NOEC ≥ 8000 µg/g	NOEC ≥ 86x maximum pollen level	Palmer and Krueger, 1999a
Parasitic Hymenoptera (*Nasonia vitripennis*)	Purified protein	NOEC = 400 µg/ml	NOEC > 4.3x maximum pollen level	Palmer and Krueger, 1999b
Earthworm (*Eisenia fetifa*)	Purified protein	NOEC = 57 mg/kg	NOEC ≥ 4.3x MEEC in soil	Hoxter *et al.*, 1999

a: NOEC - no observable effect concentration, b: MEEC - maximum expected environmental concentration

No adverse effects were observed at the maximum expected environmental concentration (MEEC) to which these nontarget beneficial organisms would be exposed. The MEEC for organisms feeding on corn plants is predicted to be 93 µg/g based on the highest level of Cry3Bb1 found in leaf and pollen tissue of MON 863 corn (Dudin *et al.*, 2001). The MEEC for soil-dwelling organisms is predicted to be 13.3 mg/kg based on an assumption that corn plants are tilled into the top six inches of soil at the time of maximum Cry3Bb1 concentration (*i.e.*, 93 µg/g). The MEEC for aquatic organisms is predicted to be 0.016 µg/l based on the following assumptions: the pollen concentration of Cry3Bb1 is 93 µg/g, the edge of field deposition rate for pollen is 0.02-0.03 mg/cm^2, and that the pollen drifts into a body of water 2 m deep.

These studies demonstrate that Cry3Bb1 proteins pose no significant risk for harm to nontarget organism populations. In all studies conducted, a NOEC was established and found to exceed predicted maximum environmental concentrations. Where possible, the NOEC was compared directly to the MEEC and found to exceed it by 4.3 to 141-fold, clearly demonstrating an adequate margin of safety for these organisms. Where it was not possible to make this direct comparison, for example in the pollen and grain feeding bioassays, it is reasonable to assume that the absence of adverse effects following exposure to a diet comprised largely of pollen or grain is indicative of no significant risk. The first year's results from a two-year field monitoring study corroborate these laboratory findings. The abundance of prominent beneficial nontarget invertebrate species was found to be comparable in conventional and MON 863 corn fields, and in some cases their abundance was higher than in fields managed with synthetic insecticides (Bhatti *et al.*, 2001).

An assessment of endangered species risk indicates that Cry3Bb1 protein would only present a hazard to terrestrial beetles of the order Coleoptera. As described above, Cry3Bb1 protein is essentially nontoxic to noninsect species, thus it poses no risk to endangered mammals, birds, noninsect aquatic organisms and noninsect soil dwelling organisms (Duan *et al.*, 2002). Cry3Bb1 has shown a high degree of specificity among insects it affects. 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. There are no endangered beetles in the Chrysomelidae family; therefore, no adverse effects on endangered beetles are expected.

The results of an aerobic soil degradation study demonstrate that Cry3Bb1 dissipates very quickly in the environment (Martin *et al.*, 2000). Analysis of soil Cry3Bb1 concentration by insect bioassay and enzyme linked immunosorbant assay (ELISA) methods established DT_{50} estimates of 2.37 and 2.76 days, respectively, and DT_{90} estimates of 7.87 and 9.16 days, respectively. The rapid dissipation of Cry3Bb1 ensures exposure risk for soil dwelling organisms will be minimal.

The results of environmental fate, field monitoring and nontarget organism toxicity studies support a conclusion that Cry3Bb1 proteins present in transgenic corn poses no significant risk to the environment.

VI. Benefits of Corn Event MON 863

There will be many benefits associated with the commercialization of corn varieties containing event MON 863. Recipients of these benefits will be growers, consumers and the environment. It is Monsanto's intention to offer this trait in combination with a low rate of a seed-applied insecticide. Cry3Bb1 will protect the root structure from corn rootworm larval feeding and the seed treatment will provide control of other soil pests that are of secondary economic importance in corn crops (*e.g.* wireworms, white grubs, and seed corn maggot). This product combination will provide growers with a complete 'in the bag' solution for management of soil insect pests; the product will have a technical fit for all corn rootworm infested acres. Varieties containing event MON 863 may also be stacked through conventional breeding with other genetically enhanced corn varieties, such as those with herbicide tolerance or insect protection.

Corn hybrids containing event MON 863 are more efficacious than soil-applied insecticides in protecting roots from larval feeding damage. The Cry3Bb1 toxin is root incorporated, it does not require activation, and its performance is unlikely to be impacted by severe environmental conditions. Superior performance and consistency of control are expected to result in a significant yield advantage for growers planting MON 863 hybrids. Preliminary estimates place this yield benefit at 1.5-4.5%. For a reasonable range of prices and yields, the value of this yield benefit to growers is $4-$12/ac relative to the use of a soil-applied insecticide, depending on corn rootworm pressure (Mitchell, 2002).

MON 863 hybrids will also provide growers with tremendous operational benefits. For corn, early planting usually results in a longer growing season and a yield benefit. However, early planting can result in insecticide performance failures because of chemical dissipation prior to larval hatch. Root protection for MON 863 hybrids will not diminish with early planting. In addition, growers will be able to plant their crop in a shorter period of time because there won't be a need to continually stop and refill insecticide boxes. Reducing the time required for growers to complete the planting operation increases the likelihood that the crop can be planted during optimal weather conditions.

Cry3Bb1 is far less hazardous than all insecticide active ingredients currently registered for corn rootworm control. The adoption of MON 863 hybrids provides an opportunity to tremendously reduce the occupational and environmental risks associated with the manufacture, transportation, storage, handling, application and disposal of conventional chemical insecticides. In year 2000, 7,835,995 pounds of insecticide active ingredient were applied to 14,197,012 acres of corn for control of corn rootworm (refer to Table 3). Numerous factors, including competitive product offerings, market acceptance, export restrictions, and hybrid availability, will influence grower decisions to replace insecticide applications with plantings of hybrids containing event MON 863. At product maturity, this technology has the potential to annually eliminate the use of millions of pounds of high risk chemical insecticides that are of concern to growers, consumers and EPA.

VII. Public Interest Factors

Registration of Cry3Bb1 and the genetic material necessary for its production in corn event MON 863 can be presumed to be in the public interest because it meets criteria for a conditional registration as delineated in the EPA policy notice regarding approval or denial of applications for conditional registration of pesticide products (EPA, 1986). This criterion states that use of a new pesticide during the period of its conditional registration must be in the public interest. Many factors can be considered in determining whether this public interest criterion has been satisfied. Registration of a new pesticide is presumed to be in the public interest if it is a replacement for another pesticide that is of continuing concern to EPA. Pesticides of concern to EPA are those which have been determined, through the special review process, to present relatively high risk, but whose registration has been continued because their benefits are relatively high. Corn containing Cry3Bb1 protein will provide an alternative to soil insecticides that are currently used for corn rootworm control and are of continuing concern to the Agency.

A. Presumption of Public Interest

The majority of insecticides registered for corn rootworm control are classified as restricted use pesticides. A listing of insecticides currently registered for corn rootworm control can be found in Table 2. The restricted use classification, imposed due to adverse environmental effects under normal use practices [40 CFR 152.171(a)], limits the use of these chemicals to certified pesticide applicators who have received special training needed for safe handling and application of these products. Additional personal protective equipment to reduce occupational exposure and special record keeping are required for restricted use pesticides.

Some of these products have been placed under EPA Special Review as described at 40 CFR §154. The Special Review process is utilized when EPA has reason to believe that the continued use of a pesticide may result in unreasonable adverse effects to people or the environment. Dimethoate, used for adult corn rootworm suppression, was subject to Special Review due to concerns about carcinogenicity, mutagenicity, fetotoxicity, and reproductive effects (EPA, 1998a). As a result of the Special Review process, certain applications of dimethoate were cancelled and labeling was modified. Granular products containing phorate and terbufos were subject to Pre-Special Review for ecological adverse effects, and are currently being evaluated for reregistration (EPA, 1998a).

In recent years EPA has adopted a quotient method for evaluating pesticide risks to nontarget organisms. Applying this method, risk quotients (RQ) are computed by comparing estimated concentrations of the pesticide in the environment to results from ecological toxicity studies with a variety of nontarget organisms. Risk quotients are compared to levels of concern (LOCs) established by EPA. The LOCs are criteria used by EPA to indicate potential risk to a nontarget organism. A finding of concern results when a RQ exceeds a level of concern. For corn rootworm insecticides that have gone through the reregistration process, risk quotients have been found to exceed EPA levels of concern.

Terbufos presents high acute and chronic risks to nontarget terrestrial wildlife species. Both avian and mammalian risks exceed the Agency's LOC (EPA. 2001b). Terbufos is the leading cause of fish kill incidents reported to EPA for any pesticide applied to corn. Phorate is highly toxic to birds and small mammals when applied at label rates. The RQ values for terrestrial animals exceed the acute risk level of corn for all species, crops and application rates (EPA, 1999a). Several bird kills, some involving large numbers of birds, have been linked to phorate use. Phorate is also highly toxic to fish and aquatic invertebrates. All phorate acute risk quotients exceed EPA high risk criteria and most chronic risk quotients exceed levels of concern. Risk quotients for chlorpyrifos indicate that a single application may pose high risks to small mammals, birds, fish and aquatic invertebrate species for nearly all registered outdoor uses (EPA, 2000a). Diazinon is highly toxic to avian and other nontarget species. Risk mitigation efforts such as lowered application rates and added label warnings have failed to substantially reduce the frequency of reported bird kills (EPA, 2000b). EPA has concluded that all uses of dimethoate may affect endangered mammals and the risk level indicates they should be considered for restricted use classification (EPA, 1999b). The Agency has concluded that all labeled rates of ethoprop result in high risks to all terrestrial and aquatic animals, except for turf slit-placement uses (EPA. 1998b). Given the extent and magnitude of the LOCs being exceeded, EPA has concluded that risks from the continued use of ethoprop can not be effectively mitigated. EPA is generally concerned about the ecological effects to terrestrial wildlife and aquatic organisms posed by exposure to methomyl (EPA. 1998c). Methomyl use presents varying levels of concern regarding avian and mammalian risk from multiple applications at short intervals. In addition, most agricultural uses present acute and chronic risk of varying levels to endangered and nonendangered aquatic organisms. At currently used rates of methyl parathion, endangered species LOCs are exceeded for all species except plants. EPA has concluded that the use of methyl parathion poses significant risk to nontarget organisms in terrestrial and aquatic environments (EPA, 1999c).

Amendments to FIFRA, passed as part of the Food Quality Protection Act of 1996, define a more rigorous safety standard for registration of pesticides used in or on food crops: this "reasonable certainty of no harm" replaces the previously used "no unreasonable adverse effects" standard. FQPA requires that EPA reassess all existing food tolerances under the new safety standard within ten years and that those pesticides posing the greatest risk to humans be given the highest priority. All organophosphate and carbamate insecticides have been placed in this highest priority grouping.

Virtually every active ingredient contained in products currently being used for corn rootworm control meet one or more EPA triggers for concern: 1) subject to EPA Special Review; 2) environmental risk quotients exceed EPA levels of concern; 3) highest priority for tolerance reassessment under FQPA; and 4) restricted use classification. A tabulation of the triggers met for each active ingredient can be found in Table 8. Cry3Bb1 and the genetic material necessary for its production in corn event MON 863 meet none of these EPA triggers for concern. Thus, the product clearly fulfills established criteria for a presumption of public interest. Consideration of the additional factors discussed in the EPA policy notice regarding conditional registration of new pesticides (EPA. 1986), further validate this presumption of public interest. These factors are discussed in the sections below.

Table 8. Indicators of EPA concern for insecticides currently registered for corn rootworm control. Collectively, these active ingredients account for 98.2% of the total insecticide applied in year 2000 for corn rootworm control.

Active Ingredient	Special Review	RQ > LOC †	Top Priority under FQPA	Restricted Use Pesticide
λ-Cyhalothrin (SP)		na		√
Carbofuran (C)	√	na	√	√
Chloroethoxyfos (OP)		na	√	√
Chlorpyrifos (OP)		√	√	
Cyfluthrin (SP)		na		√
Diazinon (OP)		√	√	√
Dimethoate (OP)	√	√	√	√
Esfenvalerate (SP)		na		√
Ethoprop (OP)		√	√	√
Fipronil (PP)		na		√
Methomyl (C)		√	√	√
Methyl parathion (OP)		√	√	√
Phorate (OP)	√	√	√	√
Tebupirimfos (OP)		na	√	√
Tefluthrin (SP)		na		√
Terbufos (OP)	√	√	√	√

† RQ – risk quotient; LOC – level of concern; na – recent environmental risk assessment not available

B. Factors Affecting a Public Interest Finding

The 1986 policy notice regarding conditional registrations indicates that EPA will consider a variety of factors pertaining to the need for a new pesticide active ingredient, its comparative benefits, risks and costs (EPA, 1986). A consideration of these factors as they relate to Cry3Bb1 corn clearly demonstrates that registration will be in the public interest.

1. Need Factors

It was hypothesized by Melhus *et al.* (1954) that the western corn rootworm has been a pest of corn in Central America for 5,000 years. Smith and Lawrence (1967) speculated that the corn rootworm became an important pest in North America when the Spanish introduced the European system of corn production. Scientific citations regarding the corn rootworm date back to the 1800s, thus reflecting the long-standing importance of this pest complex to corn production. It is by far the most significant insect pest in the U.S. Corn Belt.

Growers have historically limited economic loss caused by corn rootworm through crop rotation or the use of chemical insecticides. Crop rotation has been the primary rootworm control measure. However, the corn rootworm has shown a remarkable ability to adapt to this agronomic practice. Spread of the extended diapause and soybean variants has dramatically diminished the effectiveness of crop rotation as a pest control practice. These recent changes in corn rootworm behavior and biology suggest that the frequency of larval infestation is likely to increase in the absence of effective new control measures. This will result in an increased demand for efficacious corn rootworm control products.

Grower preference for continuous corn production in many situations, along with the adaptation of rootworm populations to rotation, have increased grower reliance on chemical control options. A thorough review of soil insecticide evaluation protocols and application equipment is described elsewhere (Mayo, 1986). The majority of growers (>90%) opt to apply a soil insecticide at planting for larval control due to greater efficacy and ease of application. Several soil insecticides can be applied post-planting at cultivation (Gray and Steffey 1998b). However, few growers rely upon a cultivation rescue treatment due to a lack of proper application equipment, laborious sampling procedures for larvae, and concern over soil moisture at cultivation time. Most of the soil insecticides may be applied only once during the growing season.

Approximately 8% of growers use corn rootworm adult management programs. Broadcast insecticide applications to suppress adult oviposition are common in the western Corn Belt, especially in Nebraska where there is an infrastructure for scouting of adult beetles by crop consultants. Cases of insect resistance to insecticides sprayed for adult control have been documented (Meinke *et al.*, 1997 and 1998).

The application of soil-applied insecticides in first year corn has increased significantly in east central Illinois and northern Indiana since 1995. The soybean variant of the western corn rootworm is spreading rapidly beyond this region towards the east; some estimate at a rate of 10 to 30 km/year (David W. Onstad, personal communication). Soil insecticide use can be expected to increase proportionally. The number of aerial applications of insecticides to soybean fields to suppress western corn rootworm oviposition may also increase during this time period even though current extension recommendations discourage this practice (Gray and Steffey 1998b).

There are many insecticides available to growers for the control of both larval and adult corn rootworms. The majority of these products are classified as restricted use and virtually all are of ongoing concern to EPA due to environmental risks. As FQPA tolerance re-assessments for the organophosphate and carbamate insecticides move towards completion, there may be fewer products available to growers.

Host plant resistance to corn rootworm has been the focus of a great deal of research with little success to date. Commercial hybrids exhibit a range of responses to larval feeding damage. Those hybrids that perform best do so because of greater root mass and a greater capacity for regrowth following root pruning. No conventional corn resistant hybrids are currently available (Gray and Steffey, 1998a).

In summary, corn growers in the U.S. have had to actively control the corn rootworm pest for over 100 years. The routine use of crop rotation and soil insecticides in modern corn production attest to the ongoing need for corn rootworm control. The effectiveness of crop rotation is decreasing due to the economics of corn production and changes in pest biology. Regulatory restrictions and a potential reduction in the number of chemical control options will increase the need for alternative corn rootworm control measures. These observations support a determination that registration of Cry3Bb1 is in the public interest.

2. Composition Factors

The composition of corn containing event MON 863 is fundamentally different from the composition of conventional insecticide end-use products. The active ingredient, Cry3Bb1, is plant incorporated. It is safer than every currently registered corn rootworm insecticide product. This characteristic of the product virtually eliminates the occupational risk currently associated with the application of chemical controls for corn rootworms. Registration of this product also provides EPA with an opportunity to reduce the manufacture, transportation, storage and disposal of millions of pounds of hazardous chemicals annually. These product characteristics support a conclusion that registration of Cry3Bb1 is in the public interest.

3. Usage Factors

The safety, convenience and simplicity of planting MON 863-containing hybrids compared to the application of conventional insecticides, along with the opportunity to extract an economic benefit through increased crop yield, are expected to make this product attractive to growers. A survey of Iowa corn producers found that 29.5% were enthusiastic and 43.6% were cautiously optimistic about the prospect of planting genetically modified hybrids that were resistant to corn rootworm larval feeding damage (Pilcher and Rice, 1998). This relatively high level of interest was based on growers' perception that such hybrids would reduce the insecticide burden in the environment (40.5% of respondents) and would lessen farm worker exposure to insecticides (21.2% of respondents). This high level of grower interest provides further evidence that registration of Cry3Bb1 is in the public interest.

4. Performance Factors

Three years of extensive efficacy field trials, conducted at multiple locations under varying levels of corn rootworm pressure, have conclusively demonstrated the superior protection of roots provided by hybrids containing event MON 863 compared to hybrids treated with conventional insecticide products. Cry3Bb1 possesses a number of unique properties that conventional insecticides do not. The protein is efficacious via a mode of action that is relatively selective to coleopteran insects. The protein is expressed throughout the roots of the corn plant. This ensures protection where it is needed and eliminates the risk of insecticide failures associated with early planting, misapplication, or unfavorable environmental conditions. Furthermore, the delivery of Cry3Bb1 in the corn seed and its production in plants eliminates many risks associated with conventional insecticide usage, some of which include improper calibration and maintenance of application equipment, handling of hazardous chemical insecticides, container disposal, chemical misplacement, runoff, and spray drift.

Integrated pest management (IPM) in agriculture includes insect scouting or monitoring to determine pest populations, consideration and application of compatible alternative biological, cultural, mechanical and chemical controls, and the establishment of action thresholds for agricultural inputs. The delivery of pest management interventions on target and on time is a key to successful IPM. Planting of MON 863 hybrids provides much greater accuracy of application compared to chemical treatments due to the localization of Cry3Bb1 within the plant tissues. Timing of application is not a factor with MON 863 hybrids since Cry3Bb1 is present in the roots throughout the growing season. Planting of MON 863 hybrids is fully compatible with current adult beetle scouting and monitoring programs that provide data upon which to base the following year's management decisions. The product is also fully compatible with cultural control measures such as crop rotation. MON 863 fits seamlessly into the concept of integrated pest management for corn. Superior protection of corn roots and a seamless fit with IPM programs indicate that registration of Cry3Bb1 is in the public interest.

5. Risk Factors

A standard battery of mammalian toxicity studies failed to provide any evidence of Cry3Bb1-induced adverse effects. The protein is rapidly degraded in mammalian digestive systems and it bears no amino acid sequence similarities to known toxins and allergens. The margin of exposure for human dietary consumption exceeds five orders of magnitude. Since the insecticidal protein is plant-incorporated, the opportunity for exposure when handling and planting seed is minimal. Planting of hybrids containing event MON 863 will essentially eliminate the occupational health risks currently associated with chemical controls for corn rootworm pests.

The selectivity of the toxin for the targeted pest minimizes risk for nontarget organisms. All currently registered corn rootworm insecticides display a broad spectrum of activity and present significant risks to a multitude of nontarget terrestrial and aquatic species. Virtually every one of these products is currently of concern to growers, consumers and the EPA for one or more reasons.

The introduction of this technology has the potential to reduce applications of conventional insecticides by millions of pounds annually. These facts indicate that registration of Cry3Bb1 is in the public interest.

6. Economic Factors

Preliminary data indicate that growers will derive a yield benefit from planting MON 863 hybrids because of the superior root protection that Cry3Bb1 provides. This yield benefit has been conservatively estimated to be 9 to 28% relative to no pest control and 1.5 to 4.5% relative to control provided by leading soil insecticides. The corn rootworm-protected trait will be priced competitively with current rootworm soil-applied insecticide products (refer to Table 5). This equates to an economic benefit for growers of \$25-\$75/ac relative to no pest control and \$4-\$12/ac relative to control provided by a soil insecticide. This preliminary economic assessment does not take into consideration potential financial gains associated with the operational benefits this technology will provide. Expenses associated with the maintenance and calibration of insecticide application equipment, and pesticide application, storage, transportation and disposal are expected to decrease. The economic risk associated with inconsistent insecticide performance is also expected to decrease. Although difficult to quantify, the flexibility this technology provides in the timing of planting is expected to result in an economic gain for the grower. The economic impact of these benefits is currently being evaluated.

Ultimately, some portion of the economic gain derived by growers using this new technology will be passed along to consumers in the form of lower commodity prices. These economic benefits indicate that registration of Cry3Bb1 and the genetic material necessary for its production in corn event MON 863 are in the public interest.

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