D. Insect Resistance Management.

BACKGROUND

Corn expressing the Cry3Bb1 protein provides protection against certain species of the corn rootworm (CRW) including the western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*) and Mexican corn rootworm (*Diabrotica virgifera zea*). In order to delay the onset of insect resistance to Cry3B1 corn, an acceptable insect resistance management (IRM) plan is necessary. The current insect resistance management (IRM) plan consists of: 1) a 20% structured refuge placed adjacent to or within the YieldGard Rootworm corn (MON 863) field, in-field strips must be at least four rows wide; 2) a resistance monitoring program, 3) a remedial action plan, and a 4) IRM grower compliance and education program. EPA also required that Monsanto provide to the Agency annual resistance monitoring, compliance (and education), and sales reports. MON 863 was registered for commercial use for the 2003 growing season and is targeted against corn rootworm (CRW, *Diabrotica* spp.).

MON 863 Cry3Bb1 Corn

Monsanto submitted several documents in support of their interim Cry3Bb1 IRM plan. An IRM plan for MON 863 corn dated June 20, 2000 was submitted to the Agency (MRID No. 451568-05). This submission included information on dose, CRW biology, simulation models of resistance development, and grower surveys. Research reports and results of grower surveys were also included in the Appendices of the June 2000 submission. An amended IRM plan dated January 8, 2002 was submitted to the Agency for review (MRID No. 455770-01). The amended plan titled "An Interim Insect Resistance Management Plan for Corn Event MON 863: A Transgenic Corn Rootworm Control Product" was intended to supercede MRID No. 451568-05. Therefore, MRID No. 451568-05 was used for additional information and as reference material, but was not formally reviewed. An additional preliminary research report dated February 20, 2001 was submitted to the Agency by Monsanto (MRID No. 453484-01).

A FIFRA Scientific Advisory Panel (SAP) was convened in August 2002. The August 2002 SAP comments regarding Monsanto's interim IRM plan were documented in a memorandum from Paul Lewis to Marcia Mulkey dated November 6, 2002 (http://www.epa.gov/scipoly/sap/2002/August/august2002final.pdf). In response to the SAP, Monsanto submitted additional information to EPA in a document from Dennis Ward to Janet Andersen dated December 13, 2002. This additional information, along with additional clarifications provided to the Agency by Dr. Michael Caprio on December 20, 2002, Dr. David Andow on December 23, 2002 and Dr. Fred Gould on February 12, 2003 was incorporated into the final review and memorandum of February 14, 2003 from Robyn Rose to Mike Mendelsohn.

Subsequent to the registration in 2003, Monsanto was required to submit resistance allele frequency data and additional IRM research data to increase the understanding of corn rootworm biology and other factors related to resistance management. The following areas of research were to be addressed.

- Research regarding adult and larval movement and dispersal, mating habits, ovipositional patterns, number of times a female can mate and fecundity;
- Research to determine if IRM strategies designed for WCRW and NCRW are appropriate for MCRW;
- Research regarding the mechanism of potential resistance of CRW to MON 863 is necessary to develop an appropriate long-term IRM strategy. Monsanto must attempt to develop resistant CRW colonies to aid in determining selection intensity;
- Research regarding the effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy needs further investigation;
- Detailed summaries of the four data-sets identified in Monsanto's December 13, 2002 letter should be submitted to the Agency to support their conclusion that the initial resistance allele frequency is = 0.01 (Reviewed by BPPD and found to be "acceptable" (Reynolds, 2004)
- Baseline susceptibility studies currently underway should be continued for WCRW and initiated for NCRW and monitoring techniques such as discriminating dose concentration assays need to be thoroughly investigated for their feasibility as resistance monitoring tools.

Monsanto was to provide protocols for the proposed research (within 90 days of the date of registration) and a progress report by January 31, 2004. A final report was to be submitted by January 31, 2006.

Monsanto submitted 12 protocols for review (submitted May 23, 2003, no MRID#) and a progress report (submitted January 30, 2004, MRID# 461865-01) covering the first four research areas. Both the protocols and the 2004 progress report were found to be "acceptable" (Reynolds, 2004).

In April of 2005, Monsanto submitted a second progress report thus providing the Agency with an update of the ongoing insect resistance management research. This second progress report (MRID 466066-01) was found to be "acceptable" (Matten, 2006).

Summary of Final IRM Research Submisssions

Monsanto submitted fourteen studies to provide additional information on the biology and ecology of the corn rootworm pest complex. They are divided into the following subject areas: CRW larval movement (Hibbard et al., 2003; Hibbard et al., 2004; Hibbard et al., 2005), CRW adult movement (Spenser et al., 2003; Kim and Sappington, 2005), CRW hosts (Clark and

Hibbard, 2004; Oyebiran et al., 2004; Wilson and Hibbard, 2004), rotation-resistant western CRW (Rondon and Gray, 2004; Crowder et al., 2005; Onstad et al., 2003), extended diapause northern CRW (Mitchell and Onstad, 2005), and baseline susceptibility of CRW to Cry3Bb1 (Siegfried et al., 2005). Data from the CRW larval movement studies have been used to make changes to the in-field refuge strip width requirement for YieldGard Rootworm, YieldGard Plus, MON 88017, and MON 88017 X MON 810, i.e., in-field strip width was changed from at least 6 to 12 rows wide to at least 4 rows wide A list of the literature citations for the studies is provided below. Findings from these studies are incorporated into the section, "Corn Rootworm Biology and Factors Related to Resistance Management."

Clark, T. L., and B. E. Hibbard. 2004. Comparison of nonmaize hosts to support western corn rootworm (Coleoptera: Chrysomelidae) larval biology: Environ. Entomol. 33:681–689.

Crowder D W; D.W. Onstad, M.E. Gray, P.D. Mitchell, J.L. Spencer, and R.J. Brazee. 2005. Economic analysis of dynamic management strategies utilizing. Transgenic corn for control of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98:961-75.

Hibbard, B. E., D. P. Duran, M. R. Ellersieck, and M. M. Ellsbury. 2003. Post-establishment movement of western corn rootworm larvae (Coleoptera: Chrysomelidae) in Central Missouri corn: J. Econ. Entomol. 96 599–608.

Hibbard, B. E., M. L. Higdon, D. P. Duran, Y. M. Schweikert, and M. R. Ellersieck. 2004. Role of egg density on establishment and plant-to-plant movement by western corn rootworm larvae (Coleoptera: Chrysomelidae): J. Econ. Entomol. 97:871–882.

Hibbard, B. E., T. T. Vaughn, I. O. Oyediran, T. L. Clark, and M. R. Ellersieck. 2005. Effect of Cry3Bb1 expressing transgenic corn on plant-to-plant movement by western corn rootworm larvae (Coleoptera: Chrysomelidae): J. Econ. Entomol. 98:1126–1138.

Kim, K.S., and Sappington, T.W. 2005. Genetic structuring of western corn rootworm (Coleoptera:Chrysomelidae) populations in the United States based on microsatellite loci analysis 34:494-503.

Mitchell, P.D. and D.W. Onstad. 2005. Effect of extended diapause on evolution of resistance to transgenic Bacillus thuringiensis corn by northern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 2220-2234.

Onstad, D.W, D.W. Crowder, P.D. Mitchell, C.A. Guse, J.L. Spencer, E. Levine, and M.E. Gray. 2003. Economics versus alleles: balancing integrated pest management and insect resistance management for rotation-resistant western corn rootworm (Coleoptera: Chrysomelidae). J Econ. Entomol. 96:1872-85.

Oyediran, I. O., B. E. Hibbard, and T. L. Clark. 2004. Prairie grasses as alternate hosts of the western corn rootworm (Coleoptera: Chrysomelidae): Environ. Entomol. 33:740–747.

Rondon, S.I., and M.E. Gray. 2004. Ovarian development and ovipositional preference of the western corn rootworm (Coleoptera: Chrysomelidae) variant in east central Illinois. J. Econ. Entomol. 97:390-396.

Siegfried, B.D., T.T. Vaughn, and T. Spencer. 2005. Baseline susceptibility of western corn rootworm (Coleoptera: Crysomelidae) to Cry3Bb1 Bacillus thuringiensis toxin. J. Econ. Entomol. 98:1320-1324.

Spencer, J.L., T.R. Mabry, and T.T. Vaughn. 2003. Use of transgenic plants to measure insect herbivore movement. J. Econ. Entomol. 96:1738-49.

Spurgeon, D.W., J.F. Esquivel, and C.P.-C. Suh. 2004. Population patterns of Mexican corn rootworm (Coleoptera: Chrysomelidae) adults indicated by different sampling methods. J. Econ. Entomol. 97:687-694.

Wilson, T. A., and B. E. Hibbard. 2004. Host suitability of nonmaize agroecosystem grasses for the western corn rootworm (Coleoptera: Chrysomelidae): Environ. Entomol. 33:1102–1108.

CORN ROOTWORM BIOLOGY AND FACTORS RELATED TO RESISTANCE MANAGEMENT

Pest Biology

In order to develop an appropriate IRM strategy for MON 863 corn, as well as all insect-protected transgenic crops, it is important to consider the biology of the target pest. Knowledge of pest biology is imperative in determining optimal size and placement of refuges that will encourage random mating between pests in Bt and non-Bt corn fields. Based on the movement of CRW adults, a non-Bt corn refuge should be planted adjacent to or within MON 863 fields.

Characteristics of pest biology that are relevant to IRM (e.g., movement, feeding habits and ovipositional habits) differ for WCRW and NCRW. WCRW and NCRW adults will feed on corn silks, pollen and young kernels in the ear tip; however, only WCRW feed on leaves. Since NCRW adults don't feed on corn leaves, they leave the field after pollination to find a field with pollen available (Branson and Krysan 1981). Since adult and larval CRW feed on various parts of the corn plant, both life stages may be exposed to the Bt protein and extended selection pressure may result (Meinke *et al.* 2001). Severe root damage from larval feeding will lead to plant lodging (where damaged corn stocks fall over making mechanical harvesting impossible) and yield losses.

WCRW and NCRW are univoltine in most of the Corn Belt (Branson and Krysan 1981, Meinke *et al.* 2001). CRW typically oviposit where the adults are feeding which is almost exclusively in corn fields (Branson and Krysan 1981, Levine and Oloumi-Sadeghi 1991). In general, CRW adult emergence varies based on species, geography, weather, management practices such as insecticide use, population density and sex. For instance, males typically emerge before females and emergence, as well as fecundity, longevity and egg viability, are reduced in corn planted later in the season (Boetel and Fuller 1997, Levine and Oloumi-Sadeghi 1991, Meinke *et al.* 2001). It is unknown what effect corn rootworm protected transgenic corn will have on phenology, sex ratio and adult emergence patterns. Asynchronous adult emergence for Bt corn fields and non-Bt refuges may lead to nonrandom or assortative mating which may lead to an increase in the rate of resistance evolution. Nonrandom or assortative mating may also occur if Bt corn disrupts the synchrony of male and female CRW adult emergence (Meinke *et al.* 2001). Mating typically occurs within 24 to 48 hours of female adult emergence within the corn fields they emerged from or nearby (Meinke *et al.* 2001).

CRW larval movement is limited particularly in areas with low population densities (Meinke *et al.* 2001). Published and unpublished articles have reported varying distances that CRW larvae move. WCRW larvae may move from 12 to 16 inches and have been found in corn rows planted up to 40 inches apart (Suttle *et al*. 1967, Short and Luedtke 1970, Gray 1999). These studies suggest that CRW larvae hatching from eggs between rows are capable of finding and injuring corn roots regardless of row spacing. Since field corn is typically planted approximately 24 to 30 inches apart, CRW may move up to two rows according to current research. However, additional information is needed to verify the distance CRW larvae move within and between rows. In general, young CRW larvae (e.g., 1st, 2nd and sometimes 3rd instars) tend to move toward actively growing corn roots. Larval movement toward respiring, growing corn roots is probably because of their ability to detect and move toward CO_2 sources (Strnad *et al.* 1986, Gray 1999). Young larvae will feed on the distal portion of corn roots and move through the soil to feed on new, short roots as they develop into later instars (Strnad and Bergman 1987, Gray 1999). It is therefore possible that a RS heterozygous larva with a partially recessive resistance trait will begin feeding on transgenic corn roots and finish its development on adjacent non-transgenic roots which would result in a non-lethal dose of MON 863 and potential survival of that larva.

NCRW and WCRW mated adults may be very mobile and have potentially high dispersal capabilities (Meinke *et al.* 2001). However, local dispersal is more common and involves movement within or among adjacent fields; whereas, migratory dispersal over long distances occurs in a small portion of individuals and usually involves females (Meinke *et al.* 2001). Dispersal capabilities of the WCRW are greater than the NCRW. The WCRW is also a greater competitor and displaced the NCRW in Nebraska by 1980 (Hill and Mayo 1980). WCRW post-mating dispersal may be local or migratory. Published data suggests that some WCRW females may leave the field after mating to oviposit elsewhere (Coates *et al.* 1986). While sustained flights by mated female CRW are possible, movement by unmated females is limited. Knowledge of the maximum and average distance an adult CRW moves is limited. Additional research regarding adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges.

Additional information was required on various aspects of CRW pest biology as it relates to a long-term IRM strategy. Characteristics of pest biology that are relevant to IRM (e.g., movement, feeding habits and ovipositional habits) differ for WCRW and NCRW; therefore, additional information on the biology of the WCRW and NCRW are needed. According to the August 2002 SAP, the WCRW and MCRW are subspecies, therefore, a lot of the data collected on biology will relate to both species. However, the Panel concluded that data on adult mating behavior, male and female migration, and reproductive biology and fecundity of females is needed to determine if the IRM plan is suitable for MCRW. Although the SAP concluded that the same IRM strategy may be appropriate for the WCRW and NCRW, the Panel recommended additional research on the NCRW and the SAP suggested collecting data from several geographic locations of the WCRW. There are behavioral differences in WCRW populations from the western and eastern regions of their distribution so studies on aspects of pest biology such as movement should be conducted in several areas. Since the biology of the SCRW is very different from the other *Diabrotica* spp. and it is not adequately controlled by MON 863, the SCRW should not be considered.

Knowledge of the maximum and average distance adult and larval CRW movement is limited. In previous submissions (MRID Nos. 453484-01 and 455770-01), Monsanto listed and summarized studies underway at the time that related to the biology of CRW. Results of these studies as well as additional research currently underway should be submitted to EPA after they are completed. Additional research regarding male and female (mated and unmated) adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges. In addition, more information is needed on mating habits, ovipositional patterns, number of times a female can mate, and fecundity. The effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy also needs further investigation.

The August 2002 SAP identified several areas of additional research needed to fully understand CRW biology as it relates to an IRM strategy. The SAP concluded that male and female adult movement and fitness in MON 863 and non-transgenic corn should be evaluated in large-scale field studies. Data needed on movement include, but are not limited to, the distance males and females will move over time and the rate adults leave the natal field. The SAP also recommended an evaluation of "the impact of adult density on migration patterns of adults, whether a delay in male emergence from MON 863 affects male fitness and lowers their chances of mating, and whether there are sublethal effects of MON 863 on female fecundity, offspring quality and other fitness parameters." Data are also needed on the movement of NCRW male and female adults since little is currently known.

The NCR-46 (a technical committee consisting of research and extension CRW specialists and other cooperators) submitted a letter dated May 29, 2001 to the EPA that outlines additional CRW biology research. The August 2002 SAP recommended that the EPA consider the recommendations made by the NCR-46.

Additional CRW biology, ecology, and genetics data were required as conditions of the YieldGard® and YieldGard® Plus registrations. The Agency has incorporated its review of these data into this chapter.

CRW larval movement.
Larval movement data published by Hibbard et al. (2003) show that between 0.75% and 6% of larvae moved across corn rows. This represents a relatively high-end estimate of the number of larvae that cross rows. This means that much narrower in-field strips should be sufficient to provide adequate protection from sub-lethal selection caused by CRW larval movement across rows and maintain low functional recessiveness. Any increase in sublethal selection would be offset by a greater probability that potentially resistant adults emerging from the Bt corn rows would be mated by susceptible adults from the refuge row. Single-row strips would likely be too narrow and allow too much larval movement across rows to sufficiently maintain low functional recessiveness. Therefore, in-field strips of ≥4 row strips (≥6 row strips preferred for lepidopteran-protected Bt corn hybrids) would provide sufficient CRW resistance management within the field based both on the consideration of the current understanding of larval movement and selection as well as grower feasibility, practicality, consistency, and compliance.

Adult corn rootworm movement. Spencer et al. (2003) developed a new technique which used ingested transgenic corn tissue as a marker for measuring movement of adult between corn and soybean fields. This method used lateral flow strips to detect the Cry3Bb1 protein in the gut of insects that had ingested YieldGard Rootworm. Insects feeding on YieldGard Rootworm could be detected for at least 16 hours after feeding, but not 32 hours. This technique allows the impact of factors, such as temperature, precipitation, and wind speed, on short-term adult movement to be studied. Spencer et al. (2003) found that 85.3% of males and females moved ≤ 4.6 – 9.1 m/d through R2-R3 stage corn. For Cry3Bb1-positive adults that moved out of corn fields into an adjacent soybean field, 86.4% of males and 93.1% of females moved ≤ 4.6 – 9.1 m/d through soybean. Data suggest that plant-to-plant movement was motivated by a search for food and was density-dependent because plant damage was density-dependent. This technique is a tool to better estimate rates of beetle movement away from transgenic corn than currently employed techniques such as fluorescent powder mark-recapture methods and offers an opportunity to better study dispersal of CRW and other insect herbivores.

Kim and Sappington (2005) studied the population genetic structure of 10 western corn rootworm populations (595 individuals sampled) from nine U.S. states (western Texas and Kansas to New York and Delaware) based on microsatellite loci analysis. These researchers found that all populations exhibited high levels of genetic diversity, with the mean allelic diversity ranging from 7.3 to 8.6, and mean expected heterozygosity ranging from 0.600 to 0.670.

Little genetic differentiation as a whole was observed across the geographic range sampled, with a global F_{st} of 0.006. Pairwise F_{st} estimates also indicated little genetic differentiation. The researchers conclude that western corn rootworm population had not had sufficient time for substantial genetic structuring since its recent eastward range expansion from the Great Plains approximately 50 years ago.

Corn rootworm hosts. A variety of grass species were studied to determine their suitability as a refuge host for corn rootworms. Several prairie grasses and forage grasses were shown to support larval growth of the western corn rootworm larvae (Clark and Hibbard, 2004; Oyediran et al., 2004; Wilson and Hibbard, 2004) and, therefore, may serve as additional refuge to the structured non-Cry3Bb1 corn refuges. This additional source of refuge could reduce the risk of Cry3Bb1 resistance evolving.

Clark and Hibbard (2004) examined larval survivorship and growth parameters of western corn rootworm on the roots of 29 plant species comprised of maize, maize-field weeds, native prairie grasses, forage grasses, and small grain crops. Adults were recovered from five plants species in addition to maize and larvae survived at least 6 d after infestation on 27 species and 24 d on 23 plant species.

Oyediran et al. (2004) evaluated 21 prairie grass species as larval hosts of western corn rootworm. Maize and sorghum were included as positive and negative controls, respectively. Overall, adults were products from 14 of 23 species evaluated.

Wilson and Hibbard (2004) monitored larval development and survivorship of western corn rootworm on 22 plant species, including maize, maize-field weeds, and selected native prairie grasses, fence-row/forage grasses and small grain crops planted in greenhouse trials. Adults were recovered from 10 species. Larvae survived at least 14 d on 21 species and 26 d on 18 species.

The potential for rootworm larvae to move between weeds within or adjacent to a maize field could be an important factor in resistance management of transgenic-rootworm maize. The long-term implication of such movement for a low-dose transgenic event has not yet been worked out.

Rotation-resistance in western corn rootworm. Traditionally, farmers rotate the planting of corn and soybeans in a field as a means to manage corn rootworm. However, in recent years, the effectiveness of this practice has been diminished because of a soybean-variant of the corn rootworm which also deposits eggs in soybean fields. Rondon and Gray (2004) studied the oviposition patterns of the soybean variant and found that, although corn can be the preferred oviposition site among crops, similar egg densities can be found in soybean and oat stubble. These results indicate that producers who choose to rotate corn with soybean or other crops, such as alfalfa, may be at risk to economic larval injury to corn roots.

Crowder et al. (2005) modeled pest management strategies from both a biological and an economic perspective. Based on these modeling efforts, greater doses were the most effective at preventing resistance to transgenic corn with the standard management strategies. This was especially true in areas without rotation-resistant phenotypes. Returns with the dynamic adoption strategies were always similar compared with the standard strategy with a medium or greater dose. If the pest management industry can achieve a high dose of toxin, farmers can plant 80% of their cornfields to a transgenic cultivar with confidence that this strategy will be beneficial biologically and economically. Results indicate that in areas without rotation resistance, planting 80% transgenic corn (required refuge is 20%) in the continuous cornfield each year generated the greatest economic returns with a medium toxin dose or greater. Where rotation resistance was a problem, planting transgenic corn in the rotated cornfield was the most effective strategy. These results support the current IRM strategy for MON 863.

Onstad et al. (2003) also modeled management strategies for rotation-resistance over a 15-year timeframe and concluded that using corn rootworm-resistant corn was an economically valuable approach using a 2-year or 3-year rotation strategy.

Overall, these studies indicate that YieldGard Rootworm corn can be a useful tool for managing rotation-resistance. Furthermore, the existing IRM plan using a 20% refuge for YieldGard Rootworm is appropriate whether or not rotation-resistant corn rootworms are present.

Extended diapause in northern corn rootworm. Some northern corn rootworm populations have developed an extended diapause period resulting in synchronization of egg hatch with the planting of corn, thus circumventing crop rotation management strategies. Mitchell and Onstad (2005) developed a population genetics model to study the impact on current IRM strategies of northern corn rootworm populations with extended diapause. The model produced mixed results depending on various other factors such as insecticide use and farmer practices but overall showed that extended diapause tended to reduce the rate of resistance evolution. No changes to the existing IRM plan for YieldGard Rootworm corn are indicated by these modeling efforts. It is recommended that further study of the impact of extended diapause in northern corn rootworm has on IRM.

Baseline susceptibility of corn rootworm to Cry3Bb1. As part of the IRM program, corn rootworm populations are being monitored for changes in susceptibility to the Cry3Bb1 protein expressed in YieldGard Rootworm. Baseline susceptibility levels of western corn rootworm populations have been measured (Siegfried et al., 2005) and serve as the baseline for measuring any annual shifts in the susceptibility of these populations. Results indicate that the representative WCRW populations collected in 2004 are susceptible to the Cry3Bb1 toxin and that slight differences in susceptibility among the populations are due to natural variation in responses. In addition to the baseline susceptibility studies conducted on western corn rootworm, populations of the northern corn rootworm and Mexican corn rootworm are being collected as part of the required monitoring program and baseline susceptibility levels are being determined for these species. Improved methods for collecting Mexican corn rootworm adults recently have been published (Spurgeon et al., 2004) and will help in this on-going effort to monitor rootworm populations.

BPPD has reviewed the baselines monitoring data provided by Monsanto from 2001-2004 (no data available for 2003) and concluded that all representative WCRW populations are susceptible to the Cry3Bb1 toxin (expressed in MON 863) and while there are slight differences in susceptibility among the populations, this is due to natural variation in the responses to the toxin (Milofsky, 2006). Specific BPPD recommendations for the resistance monitoring program are found in Milofsky (2006).

Dose

Identifying the level of dose, as related to selection intensity, is crucial when determining size and structure of a refuge needed to delay CRW resistance to MON 863 corn. CRW feeding behavior and survival and root expression data can be used to identify the dose of MON 863. From data currently available it can be concluded that MON 863 corn does not provide a high dose for CRW control. The August 2002 SAP suggested that it is not necessary to determine the difference between a low and moderate dose. It is adequate to differentiate between high dose and non-high dose products when determining effective refuge size. Therefore, MON 863 should be characterized as a non-high dose product.

According to the August 2002 SAP, comparing measures of fitness levels of susceptible homozygotes on MON 863 and non-Bt corn would provide a good approximation of selection intensity. The SAP suggested that the first step in approximating selection intensity would be to measure efficacy of MON 863 corn against CRW larvae. However, the Panel pointed out that selection intensity based on larval efficacy may be underestimated if sublethal effects or fitness costs occur. Selection intensity based on larval survival may also be underestimated if density dependent mortality is occurring. Resistant colonies of CRW should be developed to aid in determining selection intensity.

The SAP based their determination that MON 863 is a non-high dose product on the *SS* (homozygous susceptible) survival rate. The Panel also concluded that Monsanto's artificial diet assays had deficiencies, but were adequate to determine the LC_{50} for first instar larvae, level of larval resistance and dose.

Simulation Models of Resistance

In Monsanto's three-year interim IRM plan, they recommended planting a 20% non-Bt corn refuge to delay the potential of CRW resistance to Cry3Bb1. Monsanto's conclusion that a 20% refuge would be adequate to delay resistance to MON 863 corn was based on CRW biology, Cry3Bb1 effective dose, preliminary modeling results and agronomic considerations. It was concluded in the July 23, 2002 memorandum from Robyn Rose to Mike Mendelsohn that a 20%

non-Bt corn refuge planted within or adjacent to MON 863 corn fields is expected to adequately delay the risk of CRW developing resistance to Cry 3Bb1. Monsanto's IRM interim plan and EPA's review of Monsanto's plan were addressed by the August 2002 SAP in the transmittal document from Paul Lewis to Marcia Mulkey dated November 6, 2002.

According to the SAP, the current models (Monsanto's modified Caprio model, Onstad *et al*. 2001 and Andow and Alstad 2002) show that the time to resistance does not substantially differ when the refuge size ranges from 10-25%. While the SAP agreed that resistance would not occur during an initial 3 years regardless of the size of the refuge, the majority of the Panel recommended a 50% refuge would be a desirable conservative approach since resistance would be delayed substantially longer. The SAP also stated that the amount gene frequency increases during an interim period is of greater importance than years to resistance because of the potential future impact on IRM. Since MON 863 is a non-high dose product, the Panel suggested that the potential for heritable quantitative variation and rapid evolution of resistance should be considered. In addition, the models only consider monogenic (single locus) resistance, but the SAP suggested that the models consider the potential for polygenic resistance in a non-high dose product.

Additional comments were made by the Panel regarding initial resistance allele frequency. Each of the models (Andow *et al*., Onstad *et al*., Monsanto's modified Caprio model) submitted in support of Monsanto's IRM plan designated the initial resistance allele frequency as .001. However, the Panel suggested that the initial resistance allele frequency may be as low as 0.1 in a non-high dose product. Therefore, the Panel recommended that studies be conducted to determine if the initial resistance allele frequency is less than .01 and models should be run that investigate the full range of dominance values.

Monsanto responded to the August 2002 SAP in a submission letter from Dennis P. Ward (Monsanto Regulatory Affairs Manager) to Janet L. Andersen (Director, EPA's Biopesticides and Pollution Prevention Division) dated December 13, 2002. In the December 13 submission letter, Monsanto summarized results from four data sets from research they sponsored on the efficacy of MON 863. The first and third data sets consisted of field data collected from 1999 to 2002 by 22 scientists from 15 universities located in 15 states. The second data set included data collected by Dr. Bruce Hibbard (University of Missouri) and the fourth data set is from research conducted by Dr. Blair Seigfried (University of Nebraska). According to Monsanto, results of these four data sets demonstrate that the initial allele frequency is $\leq$.01. Detailed summaries of these four data sets will be submitted to the Agency for confirmation.

The first data set looked at 7500 corn plants artificially infested with $\geq$1200 CRW eggs/plant from naturally occurring populations. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 24 CRW/plant ($24 = 1200\ (1-(1-0.01)^2)$) would be resistant and the damage rating on the Iowa scale would be 3.1. Weiss *et al.* (1985) showed that <20 CRW = 3.1 on the Iowa scale. Since the average damage recorded in the first data set was 1.6, it can be concluded that the initial resistance allele frequency is ≤0.01.

The second data set summarized by Monsanto evaluated larval survival. In this study, ≥30 larvae were recovered per non-Bt corn plant at a wide range of egg infestation rates. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 0.6 resistant larvae ($0.6 = 30(1-(1-0.01)^2)$ would occur per MON 863 corn plant. Since an average of 0.7 larvae were recovered (but not feeding normally), a ≤.01 initial resistance allele frequency can be assumed.

The third data set evaluated the number of surviving adult CRW. This data set includes several studies that infest corn plants with over 1200 eggs. Of the 1200 eggs, an average of 30 adults survived on non-transgenic corn. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 0.6 resistant adults ($0.6 = 30*(1-(1-0.01)^2)$) would occur per MON 863 plant and the damage rating on the Iowa scale would equal 3. Since damage averages 1.6 on the Iowa scale, a ≤.01 initial resistance allele frequency can be assumed.

The final data set (#4) examined 11 field collected adult female CRW populations reared in the lab. Between 134 and 489 larvae per population were examined for susceptibility to Cry3Bb1. These larvae demonstrated less than 6-fold difference between the most and least susceptible populations which is similar to or less than populations of European corn borer and corn earworm in their susceptibilities to Cry1Ac and Cry1Ab. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 2% (>20) of the larvae assayed would be resistant. Monsanto asserts that no putatively resistant large larvae were recovered at high doses which suggests no larvae survived and there was low variation (lower than with lepidopterans); therefore, a ≤.01 initial resistance allele frequency can be assumed.

Products with a resistance allele frequency ≥.01 would not have enough efficacy to justify commercialization (Bourguet *et al.* 2002, Ferre' and Van Rie 2002). If the initial resistance allele frequency were 0.1, then the efficacy of the MON 863 corn would be so poor that it would not be a marketable product. At a 0.1 initial resistance allele frequency, damage would be greater than 4.6 on the Iowa root rating scale and 0.01 would result in a 3 Iowa rating. The economic threshold in corn is a 3 on the Iowa rating scale. Monsanto has demonstrated that the average damage rating is 1.6. Since MON 863 consistently provides enough protection to result in much less than a 3 root rating, it can be concluded that the initial resistance allele frequency is ≤.01 based upon product performance.

Monsanto modified Caprio's model to include an initial resistance allele frequency of 0.01 and submitted these results in their December 13, 2002 letter to Janet Andersen. Results of running this model showed that a 20% refuge would delay resistance for approximately 7-16 years (see Fig 2 on page 13 and Fig 3 on page 14 of Monsanto's December 13, 2002 submission). For this model, SS survival was set at 0.5 and RS survival set at 0.8 which is partial dominance. Based on data collected by Monsanto and cooperators, MON 863 has been shown to control an average of 50% of the homozygous susceptible (SS) CRW. Therefore, the SS survival was designated

0.5 in the modified Caprio model.

According to Monsanto, RS survival (dominance) probably equals 0.7. Therefore, basing dominance on ≥0.8 would be considered a very conservative approach. Monsanto modeled RS survival to range from 0.5 to 1. If a RS survival of 1 (absolute worst case) were to occur and the initial resistance allele frequency is assumed to be .01, then resistance would be delayed for approximately 13 years with a 20% refuge (Fig 1 on page 8 of Monsanto's December 13, 2002 submission). If RS survival is designated 0.8, then resistance will occur in approximately 16 years. According to Dr. David Andow (University of Minnesota), RS survival ranges between 0.3 and 0.8 (personal communication with Andow on 12/23/02). Therefore, a likely case assessment would be to designate RS as 0.8 which suggests that 80% of the heterozygotes survive.

Monsanto also provided the Agency with additional runs of the modified Caprio model that included conservative parameters representing a worst case scenario. These additional models included initial resistance allele frequencies of .01 and .001, RS dominance values of 0.7 and 0.8 and SS survival ranging from 0.1 to 0.8. Results of the model incorporating these conservative input parameters (e.g., initial allele frequency = .01; RS dominance value = 0.8; SS survival = 0.1) suggested that CRW resistance to Cry3Bb1 will not occur for at least seven years assuming 100% MON 863 market penetration and 100% IRM compliance (Table 1).

Table 1. Predictions for MON 863 durability with a 20% refuge

SS Survival	RS Dominance	Allele Frequency = .01	Allele Frequency = .001
0.1	0.7	7 years	9 years
0.1	0.8	7 years	9 years
0.3	0.7	11 years	15 years
0.3	0.8	10 years	13 years
0.5	0.7	20 years	30 years
0.5	0.8	16 years	23 years
0.8	0.7	> 100 years	> 100 years
0.8	0.8	> 50 years	> 50 years

Monsanto also commented on the SAP's recommendation to consider polygenic resistance in the simulation models. According to Monsanto, results of the model will not differ if polygenic resistance is considered rather than monogenic resistance. Dr. Mike Caprio (Mississippi State University) agreed with Monsanto's conclusion. According to Dr. Caprio applying monogenic or polygenic resistance to the models does not affect the outcome in the absence of refuge (personal communication with Mike Caprio on 12/20/02, Caprio 1998). Groeters and Tabashnik (2000) concluded "that the intensity of selection, rather than the number of loci conferring resistance, is central in determining rates of resistance evolution and effectiveness of refuges." This new information provided to the Agency by Monsanto after the August 2002 SAP suggests that assuming CRW resistance to MON 863 is polygenic rather than monogenic will not affect the results of the models.

Based on the additional information submitted to the Agency by Monsanto after the August 2002 SAP and results of running Caprio's modified model with a .01 initial resistance allele frequency, it can be concluded that a 20% refuge will delay resistance for approximately 7 to 16 years and probably longer since the model also assumes 100% adoption. However, Monsanto assumes that 50% of the susceptible homozygotes (SS) will be controlled. Efficacy data submitted thus far shows 17% to 62% larval survival on MON 863 corn. If the SS input parameter were changed to a lower level of efficacy (e.g., 0.3), then the years to resistance may decrease.

Based on the results presented in Monsanto's recent submission and recommendations from national experts, including the NCR 46, a 20% refuge should be adequate to delay resistance for 7 to 16 years. In addition, because growers are familiar with the 20% refuge required for currently registered Bt corn products, complianceis expected based on grower familiarity, feasibility and presenting a consistent message to growers. A 20% refuge should be planted adjacent to or within fields.

Resistance Allele Frequency Data, MRID No. 459438-01.
Greenhouse and field efficacy studies, adult emergence trials and laboratory feeding studies have generated data for estimating initial resistance allele frequency in corn rootworm populations feeding on transgenic MON 863 corn containing the genes for Cry3Bb1 endotoxin, as compared to isoline corn without Cry3Bb1 expression. These four studies (root damage ratings in the greenhouse and field; larval establishment in the field; adult survival in the field; and larval susceptibility to Cry3Bb1 in a laboratory bioassay) were conducted between 2000 and 2002. Monsanto and university trials, the work of Hibbard et al. and data from Siegfried and Spencer were summarized in this report. Artificial infestations (eggs) of western corn rootworm (WCRW) were used to challenge both greenhouse and field populations of MON863. Bioassay of artificial diet top-loaded with five concentrations of purified Cry3Bb protein and control were tested with larvae reared from eggs laid by adult female WCRW collected from 11 distinct field populations in 6 midwestern states. Bioassay results for the MON 863 low-to-moderate dose product exhibited a 6-fold regional variation in larval susceptibility on the basis of LC_{50} values (2.22 $\mu g/cm^2$ vs 13.00 $\mu g/cm^2$). In all four studies, predicted results based on the Hardy-Weinberg law and the assumption of an *r*-allele frequency of either 0.1 or 0.01 (for parameters of damage to MON 863 plants, number of larvae on each MON plant, and number of resistant larvae) were compared with measured observations.

The evidence presented in the four data sets is largely circumstantial -- r-allele frequency was estimated using three field efficacy studies and the results from Cry3Bb baseline susceptibility work. Major assumptions were made regarding density dependent mortality, the genetics of potential resistance (assumed to be dominant), and the mode-of-action of MON 863 corn. While these indirect approaches provide some support to Monsanto's hypothesis that the r-allele frequency for CRW is <0.01, there is too much uncertainty to definitively prove that it is definitely the case.

Monsanto has been required to continue to pursue the r-allele issue through the development of resistant CRW colonies (as is required as a separate condition of registration) and other research efforts. A better understanding of the mode-of-action (i.e. toxic or repellent effects) would also aid in the understanding of potential CRW resistance. In addition, research on dose, fitness, behavior, and possible polygenic inheritance could also be useful to further the understanding of CRW resistance.

Refuge

A 20% non-Bt corn refuge is necessary to produce an adequate number of CRW susceptible to the Cry3Bb1 protein. There are two ways a grower can implement the refuge requirement. A non-Bt corn refuge can be planted as a continuous block adjacent to the MON 863 fields or as non-transgenic strips planted within transgenic field. Considering the limited movement of CRW larvae, planting refuges close to transgenic fields in large blocks is preferred to narrow strips (Gray 1999, Meinke *et al.* 2001). If a 20% refuge is planted as row strips within a corn field, then at least 4 or more consecutive rows of non-Bt corn should be planted. (Hibbard *et al.* 2003). Use of an in-field strip refuge is not intended for fields planted to increase inbred seed since these fields need to be isolated from external corn pollen sources. An in-field or adjacent non-Bt corn refuge would be inconsistent with inbred seed production practices.

Soil applied insecticides to control CRW larvae are acceptable on refuge acres. The ability to treat refuges with larval insecticides is necessary to avoid the potential for severe damage and economic impact. However, it is not acceptable to treat refuges for adult CRW control since these treatments may diminish the effectiveness of the refuge. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (Bt and non-Bt) should be treated identically.

Bt fields and the non-Bt refuge acres should be treated with identical agronomic practices such as irrigating all corn (Bt and non-Bt) at the same time. To ensure the production of similar numbers of CRW, Bt and non-Bt corn should be planted in fields with similar backgrounds. For example, if MON 863 hybrids are planted on continuous corn fields then the non-Bt refuge should be planted on continuous corn fields or both should be planted on first-year corn acres. Likewise, non-Bt refuges should be planted on first year corn fields if the MON 863 hybrids are planted on first year corn fields.

Monitoring for Resistance

A resistance monitoring strategy for Bt corn is needed to test the effectiveness of resistance management programs. Detecting shifts in the frequency of resistance genes (i.e., susceptibility changes) through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs. As such, the utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. Monitoring techniques such as discriminating dose concentration assays need to be thoroughly investigated for *Diabrotica* spp. for their feasibility as resistance monitoring tools.

Grower participation (e.g., reports of unexpected damage) is an important step in resistance monitoring. Resistance monitoring is also important because it provides validation of biological parameters used in models. However, resistance detection/monitoring is a difficult and imprecise task. It requires both high sensitivity and accuracy. Good resistance monitoring should have well-established baseline susceptibility data so changes in pest susceptibility over time can be monitored. Baseline susceptibility data for WCRW and NCRW to Cry3Bb1 have been collected, but no diagnostic concentration or discriminating dose has yet been determined. These data are also needed for MCRW.

A comprehensive monitoring plan that targets the CRW and addresses when and where monitoring will occur is needed and should be developed within two years of commercialization. The August 2002 SAP recommended a two tiered approach to monitoring for CRW resistance to MON 863. The Panel recommended tier 1 monitoring methods should identify locations that would merit tier 2 laboratory bioassays. Early detection monitoring should be directed to areas with the highest rate of MON 863 adoption since these areas represent the highest risk of resistance occurring.

The August 2002 SAP suggested that current methods used for early detection of resistance probably do not have the necessary level of sensitivity. Therefore, the Panel recommended potential alternatives to the insect bioassay using artificial diet. For instance, susceptibility of neonate larvae to corn lines expressing varying levels of the Cry3Bb1 protein (e.g., events MON 863, MON 862, MON 853 and MON 854). Measuring larval mortality and growth data with various corn lines rather than artificial diet would be easier and may eliminate some of the problems associated with the feeding bioassay such as mold growth on the artificial diet. Susceptibility data should also be collected for the NCRW and MCRW.

The SAP also suggested that data on root damage may be used as a monitoring tool. However, a method of using root damage ratings to monitor for resistance has not been developed or validated at this time. It also may be possible to use data on emergence patterns in the MON 863 and non-Bt corn refuges. More females than males from susceptible populations tend to emerge from MON 863. It may be possible to evaluate the percentage of males emerging and be correlated with resistance.

Monitoring will become more important after the accrual of multiple growing seasons of exposure and grower adoption increases. In addition to baseline susceptibility data, information is needed to determine how many individuals need to be sampled and in how many locations. The chance of finding a resistant larva in a Bt crop depends on the level of pest pressure, the frequency of resistant individuals, the location and number of samples that are collected, and the sensitivity of the detection technique. Therefore, as the frequency of resistant individuals or the number of collected samples increases, the likelihood of locating a resistant individual increases (Roush and Miller 1986). If the phenotypic frequency of resistance is one in 1,000, then more than 3,000 individuals must be sampled to have a 95% probability of one resistant individual (Roush and Miller 1986).

Remedial Action

The initial observation of unexpected CRW damage or suspected resistance will likely occur by the grower. Unexpected damage will probably be observed as lodged corn plants. Growers should be required to report any unexpected CRW damage such as lodged plants to the registrant. The August 2002 SAP identified the following four steps a registrant should take to determine if further testing is needed to confirm resistance is occurring.

- "request the grower check planting records"
- "rule out damage from nontarget insects, weather, or other environmental factors"
- "conduct tests to verify MON 863 was planted and that the correct percentage of plants are expressing"
- "if plants are MON 863 and damage approaching a 0.5 (node-injury scale) is found on any expressing plant, evaluate roots from the corresponding refuge"

Resistance should be confirmed by a standard diet bioassay or evaluation of root node injury. An insect diet bioassay with the Cry3Bb1 protein that results in a LC_{50} that exceeds the upper limit of the 95% confidence interval of the LC_{50} established from baseline measurements of susceptible populations could be used to confirm resistance. Alternatively, resistance may be confirmed when one or more root nodes of at least 50% of Cry3Bb1 plants grown in the laboratory are destroyed. A discriminating concentration bioassay may also be used to confirm resistance; however, this method may take a long time to develop. The August 2002 SAP also recommended investigating the potential of using samples of populations surviving on Bt corn or an evaluation of larval root tunneling to confirm resistance.

Confirmed resistance should be reported to EPA as soon as possible and must be within 30 days. Once resistance has been confirmed, alternative control measures to reduce or control the local target pest population should be recommended to customers, extension agents, consultants, university cooperators, seed distributors, processors, state regulatory authorities, EPA regional and national authorities, and any other pertinent personnel of the incidence(s) of resistance in the affected area. Where appropriate, customers and extension agents in the affected area should apply insecticides and/or crop rotation practices to control any potentially resistant individuals.

As soon as possible following confirmation of resistance, but within 90 days, Monsanto should notify the Agency of the immediate mitigation measures that were implemented and submit a proposed long-term resistance management action plan for the affected area. Monsanto should work closely with the Agency in assuring that an appropriate long-term remedial action plan for the affected area is implemented. A remedial action plan that is approved by EPA should be implemented that consists of some or all the following elements, as warranted: 1) Inform customers and extension agents in the affected area of pest resistance; 2) Increase monitoring in the affected area, and ensuring that local target pest populations are sampled on an annual basis; 3) Recommend alternative measures to reduce or control target pest populations in the affected area; 4) Implement intensified local IRM measures in the affected area based on the latest research results. The implementation of such measures will be coordinated by the Agency with other registrants; and 5) Monsanto should cease sales of all MON 863 Bt corn hybrids in the affected area until resistance has been shown to have been abated. During the sales suspension period, Monsanto may sell and distribute in these counties only after obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy should be coordinated with the Agency.

For the growing season(s) following a confirmed resistance incident(s), Monsanto should maintain the sales and distribution suspension of all MON 863 hybrids potentially affected by the resistant pest populations or areas in which resistance is considered to be serious. This must be done within the affected region or if undetermined, the affected county(ies) and proximate surrounding counties. This sales suspension should remain in place until resistance has been determined to have subsided (within 5 to 10% or one standard deviation of baseline levels). In addition, Monsanto should develop, recommend, and implement alternative resistance management strategies for controlling the resistant pest(s) on corn with all necessary personnel (e.g. growers, extension agents, consultants, seed distributors, processors, university cooperators, and state/federal officials) in the affected region/county(ies) and surrounding counties of the resistance situation. All necessary personnel (e.g. growers, consultants, extension agents, seed distributors, processors, university cooperators, and state/federal authorities) in the affected region/county(ies) and surrounding counties of the resistance situation should be informed. Monitoring and surveillance in the affected area(s) for resistance and define the boundaries of the affected region should be intensified and studies on the rate of decline of resistance in the field should be conducted. Monsanto should continue to work with the Agency, states, grower groups, extension agents, consultants, university cooperators, or other expert personnel and other stakeholders to ensure the implementation and development of appropriate mitigation measures for resistance in the affected areas.

Grower Education and Compliance

Growers are perhaps the most essential element for the implementation and success of any IRM plan as they will ultimately be responsible for ensuring that refuges are planted according to guidelines and that Bt fields are monitored for unexpected pest damage. Therefore, a program that educates growers as to the necessity of IRM and provides guidance as to how to deploy IRM should be an integral part of any resistance management strategy. The 2000 SAP also suggested that a comprehensive education program may help increase IRM compliance (SAP 2001). Ideally, the educational messages presented to growers should be consistent (among different registrants if applicable for CRW) and reflect the most current resistance management guidelines. Specific examples of education tools for growers can include grower guides, technical bulletins, sales materials, training sessions, Internet sites, toll-free numbers for questions or further information, and educational publications.

To avoid confusing or discouraging growers, new IRM programs should be kept simple and consistent with existing programs so that growers will not be discourage from properly implement IRM or will not grow transgenic crops. Growers should be required to sign a technology use agreement that outlines IRM requirements and acknowledges the growers responsibility to comply with them on an annual basis. The agreement will also state that growers received the Product Use Guide. This agreement may be a section of the growers order sheet or some other document or format. An annual industry-supported survey conducted by a third party should be submitted to the Agency as a tool to monitor grower compliance. Additional education efforts should target non-compliant growers and access to the technology will be limited for growers found to be non-compliant.

YieldGard Plus / MON 863 x MON 810

Monsanto has developed an insect resistance management (IRM) plan for YieldGard® Plus Corn. An amendment to Monsanto's IRM plan in Volume 1 was submitted to EPA by Monsanto on April 1, 2003. Since YieldGard® Plus Corn contains the Cry1Ab and Cry3Bb1 proteins, an IRM plan needs to consider European corn borers and corn rootworms. Monsanto's IRM plan for YieldGard® Plus Corn considers European corn borer and corn rootworm resistance management and takes the conservative approach when strategies differ between the target pests.

Refuge Requirements. Based upon growers' agronomic practices and pesticide use, growers may plant one refuge for European corn borers and corn rootworms or separate refuges may be planted for each pest. A grower that adopts the Common Refuge option would be required to plant a minimum of a 20% non-Bt structured refuge adjacent to or within YieldGard® Plus Corn fields. Refuges acres should be planted as continuous blocks adjacent to or within fields, perimeter strips, or strips within YieldGard® Plus Corn. Monsanto is recommending that in-field strips should be at least four row and preferably six rows wide. Agronomic practices should be comparable for YieldGard® Plus Corn and refuge acres. For example, if YieldGard® Plus Corn acres are planted continuously or as first year corn, then the non-Bt refuge acres should also be planted continuously or as first year corn respectively. Non-Bt insecticides may be applied to refuge acres to control corn root larvae, but may only be applied to refuge acres when corn rootworm adults are present if YieldGard® Plus Corn acres are also treated.

Growers that choose the Separate Refuge option must plant a distinct refuge for corn rootworm and European corn borer. A 20% non-corn rootworm protected corn refuge must be planted to delay corn rootworm resistance to YieldGard® Plus Corn. An additional 20% non-Bt corn must also be planted to delay European corn borer resistance. The corn rootworm refuge must be planted with corn that does not contain the Cry3Bb protein. However, corn that only contains the Cry1Ab protein may be planted if a separate non-Bt corn refuge is planted to delay European corn borer resistance. The corn rootworm refuge should be planted as continuous blocks adjacent to or within fields, perimeter strips or strips within YieldGard® Plus Corn (at least 4 rows), and utilize comparable agronomic practices as the YieldGard® Plus Corn acres. European corn borer refuges may be planted within fields as blocks or strips, adjacent to fields or up to ½ mile (1/4 mile preferred) from YieldGard® Plus Corn acres. Non-Bt insecticides may be applied to refuge acres to control corn rootworm larvae, but may only be applied to refuge acres when corn rootworm adults are present if YieldGard® Plus Corn acres are also treated. Non-Bt insecticides may be applied to refuges to control the European corn borer, corn earworm or southwestern corn borer if economic injury levels occur.

Comparison of event MON 810 and MON 863 IRM requirements with Monsanto's proposed IRM strategy for YieldGard® Plus Corn

Requirements	**MON 810**	**MON 863**	**YieldGard® Plus Corn**
Refuge Size	20%	20%	20%
Refuge Placement	½ mile (¼ mile preferred)	Adjacent or within field	Adjacent or within field
Refuge Configuration	Block, in-field strips (4 rows, where 6 rows preferred), or edges	Block or strips (4 rows)	Block or strips (4 rows)

Requirements	MON 810	MON 863	YieldGard® Plus Corn
Refuge Management	Any corn rotation meeting placement & configuration requirements. Insecticides can be used in refuge to control ECB/SWCB when above economic thresholds. Microbial *Bt* insecticides are not allowed.	Same corn rotation as *YGRW* (*e.g.*, first year corn or corn followed by corn). Conventional insecticides or seed treatments can be used in refuge to control CRW larvae & other soil pests. If the refuge is treated with a foliar insecticide labeled for CRW control when CRW adults are present, then *YGRW* also must be treated. (Not applicable)	Same corn rotation as *YG Plus* (*e.g.*, first year corn or corn followed by corn). Conventional insecticides or seed treatments can be used in refuge to control CRW larvae & other soil pests. If the refuge is treated with a foliar insecticide labeled for CRW control when CRW adults are present, then *YG Plus* also must be treated. Microbial *Bt* insecticides are not allowed.
Refuge Corn Types	Conventional	Conventional *YGCB* (a CB refuge planted ½ mile also will be required) *Roundup Ready corn*	Conventional *YGCB* (an additional refuge for CB will be required) *Roundup Ready corn*

Grower Agreements. Growers will be required to sign an agreement similar to the agreements growers currently sign to plant MON 810 or MON 863 corn. This signed agreement contractually obligates growers to comply with refuge requirements.

Grower Education. Grower education programs are required in an IRM strategy. Monsanto will develop and implement grower education programs to inform growers of YieldGard® Plus Corn IRM requirements and report on these programs to EPA.

Compliance Assurance Plan. Monsanto is currently developing a program that will evaluate and promote grower compliance. A grower compliance plan for YieldGard® Plus Corn is required.

Monitoring. Monsanto will be required to monitor for pest resistance to the Cry1Ab and Cry3Bb1 proteins in YieldGard® Plus Corn. Monsanto will develop a plan for YieldGard® Plus Corn to monitor for changes susceptibility of the European corn borer, southwestern corn borer, corn earworm and western corn rootworm. Monsanto must develop a diagnostic concentration assay for CRW. The use of a panel of experts might useful to evaluate both the CRW and stalkborer monitoring programs in both YieldGard® and YieldGard® Plus Corn fields.

Mitigation. A Remedial Action Plan must be developed and implemented if resistance is detected. This plan will rely on the remedial action elements developed for European corn borer and corn rootworm.

Reporting. Reporting requirements for YieldGard® Plus Corn will be the same as for MON 810 and MON 863 corn. Sales reports, IRM grower agreement results, compliance and educational programs will be submitted to EPA annually by January 31. Resistance monitoring reports for European corn borer and CRW will be submitted to EPA annually.

MON 88017

The Cry3Bb1 protein expressed in MON 88017 corn is functionally and physiologically similar to that expressed in MON 863 corn. The proteins differ by only one amino acid of 653 (99.8% homology) and are expressed at comparable levels in the plant. To test for functional equivalence, Monsanto conducted susceptibility assays with Colorado potato beetles (CPB) and western corn rootworm (WCRW), as well as field efficacy tests against CRW larvae. The susceptibility assays involved diet incorporation of Cry3Bb1 from each hybrid to determine LC_{50} values for the test insects. The results were similar for both Cry3Bb1 variants: 1) for CPB, the MON 88017 variant (Cry3Bb1.pvzmir39) had an LC_{50} of 0.84 µg/ml while the MON 863 variant (Cry3Bb1.11098) had an LC_{50} of 0.95 µg/ml; 2) for WCRW, the LC_{50} was 139 µg/ml for the MON 88017 variant and 100 µg/ml for the MON 863 variant. Field efficacy trials were conducted with MON 88017, MON 863, and non-expressing control plants using artificial infestations of WCRW eggs. Efficacy was measured as protection against feeding damage using a root rating scale. Seven weeks after infestation, the root damage ratings (RDR=0.12) were identical for MON 88017 and MON 863, both of which were significantly lower than the level of damage on the control plants (RDR=1.47).

In addition to structural and functional analysis of the Cry3Bb1 toxin, Monsanto also determined protein expression levels in MON 88017 relative to those for MON 863. Using ELISA techniques, leaf, root, pollen, silk, grain, and stover tissues were analyzed for the amount of Cry3Bb1 protein both in dry weight and fresh weight tissues. The results showed that the protein expression in MON 88017 was comparable to MON 863: expression was slightly higher in young leaf, stover, and silk tissues, slightly lower in pollen, and the same in forage, forage root, and grain tissues. Only the expression in silk was significantly different. When tracked through the growing season, the amount of Cry3Bb1 protein declined in MON 88017 leaf, whole plant, and root tissue in a manner similar to that observed for MON 863.

The IRM plan developed for MON 863 corn is compatible with MON 88017 corn (MRID No. 461817-01**)** and all aspects of the MON 863 IRM plan are to be followed for MON 88017.

MON 88017 x MON 810

Monsanto's submission indicates that the Cry3Bb1 and Cry1Ab toxins expressed in MON 88017 x MON 810 are "physiologically and functionally" equivalent to that expressed in MON 863, MON 88017, and MON 810. To demonstrate the physiological equivalence, Monsanto investigated the amino acid sequences of the Cry3B1 toxins produced in both MON 88017 and MON 863. The Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of >99.8%, differing from one another by only one of 653 amino acids. Since the Cry1Ab toxin was introduced using conventional breeding with MON 810, the toxins in MON 88017 x MON 810 and MON 810 should be identical. To test for functional equivalence, field efficacy tests were conducted against CRW and ECB larvae. Four treatments were used: MON 88017 x MON 810, MON 88017, MON 810 (crossed with a glyphosate tolerant hybrid), and a non-expressing control (a glyphosate-tolerant hybrid). For ECB, evaluations natural infestations were used, which were supplemented by artificial infestations at the whorl stage. Damage (efficacy) was determined by assessing leaf damage (LDR) using the Modified Guthrie Scale (0=no damage, 9=high damage). CRW efficacy was also evaluated with artificial infestations of western corn rootworm (WCRW), which was done at the second leaf stage (V2). Damage was assessed using a root damage rating (RDR) scale (Oelson Node Injury Scale). The results for ECB efficacy (tabulated after 21 days) showed that both MON 88017 x MON 810 and MON 810 alone had low amounts of leaf damage (LDR= 0.8 and 0.9 respectively), while the MON 88017 alone and non-expressing control had significantly higher levels of damage (LDR=2.7 for both). For WCRW (determined after 6-7 weeks), both MON 88017 x MON 810 and MON 88017 alone had significantly greater root protection (RDR=0.1 for both) than MON 810 alone or the non-expressing control (RDR=1.24 and 1.35 respectively).

In addition to the structural and functional analysis of the Cry3Bb1 and Cry1Ab toxins, Monsanto also determined protein expression levels in MON 88017 x MON 810 relative to those for MON 88017 and MON 810. (MON 88017 had been previously compared with MON 863 for Cry3Bb1 expression which was found to be almost identical). Using ELISA techniques, young leaf, young root (Cry3Bb1 only), pollen (Cry3Bb1 only), forage (leaf), forage root (Cry3Bb1 only), and grain tissues were analyzed for the amount of Cry3Bb1 and Cry1Ab protein both in dry weight and fresh weight tissues. The results showed that the Cry3Bb1 protein expression in MON 88017 x MON 810 was comparable to MON 88017 in all tissues. Expression in MON 88017 x MON 810 was slightly lower in young root and grain tissues and was higher in all other tested tissues, though none of the differences were statistically significant. For Cry1Ab, expression in MON 88017 x MON 810 was also comparable to MON 810, with only slight insignificant differences in young leaf, forage leaf, and grain tissues. The IRM plan developed for Yieldgard Plus (MON 863 x MON 810), consisting of CRW (MON 863) and Lepidoptera (MON 810) components, is compatible with MON 88017 x MON 810 corn (MRID No. 461850-01) and all aspects of the Yieldgard Plus IRM plan are to be applied to MON 88017 x MON 810.

CONCLUSIONS

A 20% non-Bt corn refuge for Cry3Bb1 corn is sufficient for an additional 5 year interim period. Based on reviewed corn rootworm genetics, biology, and ecology data Monsanto submitted as required by the terms and conditions of the YieldGard® Rootworm Corn (524-528) and YieldGard® Plus Corn (524-545) registrations, as well as the published literature on these subjects we conclude that the IRM strategy plans in existence for MON 863 and YieldGard Plus, as well as those proposed for MON 88017 and MON 88017 X MON 810, are sufficient to mitigate the likelihood of rootworm resistance for now. However, since there are still uncertainties associated with the selection for rootworm resistance to the Cry3Bb1 toxins, the adequacy of the IRM plans should be reevaluated after 5 more years. The non-Bt corn refuge should be planted as continuous blocks adjacent to the MON 863 fields, as perimeter strips or, as non-transgenic strips planted within transgenic field. A 20% non-Bt corn refuge is necessary to produce an adequate number of CRW susceptible to the Cry3Bb1 protein. Considering the limited movement of CRW larvae, planting refuges close to transgenic fields in large blocks is preferred to narrow strips (Gray 1999, Meinke *et al.* 2001). If a 20% refuge is planted as row strips within a corn field, then it should be planted as at least 4 or more consecutive rows (Hibbard *et al.* 2003).

Seed and granular insecticide treatments to control CRW larvae are acceptable on refuge acres. However, it is not acceptable to treat refuges for adult CRW control these treatments may diminish the effectiveness of the refuge. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (Bt and non-Bt) should be treated identically. Bt fields and the non-Bt refuge acres should be treated with identical agronomic practices such as irrigating all corn (Bt and non-Bt) at the same time. To ensure the production of similar numbers of CRW, Bt and non-Bt corn should be planted in fields with similar backgrounds. For example, if MON 863 hybrids are planted on continuous corn fields then the non-Bt refuge should be planted on continuous corn fields or both should be planted on first-year corn acres. Likewise, non-Bt refuges should be planted on first year corn fields if the MON 863 hybrids are planted on first year corn fields.

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